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ERRATA, VOLUME II.

Page 51, Fig. 3, for constant*ine*, read *constantan.*

" 316, fourth line from bottom, for *nu*mbers, read *members.*

" 318, line 16, transpose comma to follow *days.*

" 319, line 26, for *n*ixture, read *mixture.*

" 319, fifth line from bottom, for support*s*, read *supported.*

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INVESTIGATIONS ON THE PHYLOGENY OF THE ANGIOSPERMS

5. FOLIAR EVIDENCE AS TO THE ANCESTRY AND EARLY CLIMATIC ENVIRONMENT OF THE ANGIOSPERMS

EDMUND W. SINNOTT AND IRVING W. BAILEY

The leaf of the Angiosperms is so variable in its shape and venation among closely related species and so easily modified by environmental influences that gross foliar characters have been largely neglected by the taxonomist and the phylogenist except within small groups of plants. A careful survey of evidence obtained from the various botanical fields, however, apparently makes possible a reconstruction of the primitive Angiosperm leaf with a reasonable degree of certainty, and a determination of some of the factors which have modified it; and thus suggests not alone the probable ancestry of the Angiosperms but also the climatic conditions under which they first appeared. The presentation of evidence on which such an hypothesis may be built is the purpose of the present paper.

It is with the more ancient of the two Angiosperm classes, the Dicotyledons, that the problem necessarily rests. The two main types of leaf venation in this group are the palmate and the pinnate, between which intermediate conditions frequently occur. Leaf shape is, of course, generally correlated with venation although there are numerous instances where broad leaves are pinnate and narrow ones palmate. The main leaf types, having reference both to venation and shape, may be roughly designated as the palmate simple (fig. 2),

[The **Journal** for December (**1** : 499–550) was issued 29 Dec. 1914.]

palmate lobed (fig. 3), palmate compound[1] (fig. 7), pinnate simple (fig. 1), pinnate lobed (fig. 17) and pinnate compound (fig. 10). A brief survey of the distribution of these types throughout the three main growth forms of Dicotyledons (trees, shrubs and herbs) in various regions of the earth is set forth in the following table.[2]

It will be observed that in these modern floras the pinnate type is markedly predominant everywhere and that the simple pinnate condition alone constitutes from 60 per cent to 70 per cent of the species. Other noteworthy facts are the abundance of compound leaves in warm regions, the almost complete absence there of palmately lobed woody plants, and the practical confinement of the pinnately lobed type to herbaceous forms.

In view of the preponderating evidence that woody Angiosperms are more ancient than herbacous ones,[3] it seems probable that leaf

---

[1] Trifoliate leaves with stalked terminal leaflets (frequently called pinnately trifoliate) will be regarded in this paper as palmately compound.

[2] Analyses have been made of the following floras as to types of leaf or types of node or both:

North America: Flora of the Northern United States and Canada, Britton and Brown; Flora of the Rocky Mountains, Coulter; Flora of the Florida Keys, Small; Flora of the British West Indian Islands, Grisebach; Flora Nicaraguensis, Goyena.

South America: Flora Brasiliensis, Martius and others; Historia de Chili: Botânica, Gay; Report of the Princeton Expedition to Patagonia: Botany, Macloskie.

Europe: English Botany, Sowerby; Flora des Nordostdeutschen Flachlands, Ascherson and Graebner; Flora Rossica, Ledebour; Flora des Alpes, Bouvier; Flora Española, Lazara; Flora Italica, Fiori and Paoletti.

Asia: Flora Orientalis, Boissier; Flora of Syria, Palestine and Sinai, Post; Flora Simlensis, Collett; Flora of the Upper Gangetic Plain, Duthie; Flora of Bombay, Cooke; Handbook of the Flora of Ceylon, Trimen; Maylayan Flora, King; Flora van Nederlandesch Indië, Miquel; Flora Hongkongensis, Bentham; Flora of Manila, Merrill.

Africa: Manual of the Flora of Egypt, Muschler; Flora of Tropical Africa, Oliver, Thiselton-Dyer and others; Flora Capensis, Harvey and Sonder, and others; Forests and Forest Flora of Cape Colony, Sims; Natal Plants, Wood; Plantes de Madagascar, Baillon and Castillo; Flora of Mauritius and the Seychelles, Baker.

Australasia: Flora Australiensis, Bentham; New Zealand Flora, Cheeseman.

Oceana: Flora of the Hawaiian Islands, Hillebrand; Indigenous Trees of the Hawaiian Islands, Rock.

Analyses were also made of the genera of woody Dicotyledons enumerated in Engler and Prantl's Natürliche Pflanzenfamilien; and of the species of trees and shrubs from China and Japan in the herbarium of the Arnold Arboretum.

[3] The Origin and Dispersal of Herbaceous Angiosperms. Sinnott, E. W .and Bailey, I. W., Annals of Botany, 28: Oct. 1914.

TABLE I

		Palm. S.	Palm. L.	Palm. C.	Pinn. S.	Pinn. L.	Pinn. C.
		%	%	%	%	%	%
Engler							
Tropical Regions							
Trees	1,326 genera	6	2	4	62	—	26
Shrubs	1,257 "	14	1	3	69	—	12
Temperate Regions							
Trees	273 "	8	6	5	60	1	19
Shrubs	776 "	7	2	5	76	2	8
N. United States	*2,821* sps.						
Trees	163	7	6	3	57	8	19
Shrubs	328	5	9	8	72	—	7
Herbs	2,330	11	4	10	58	6	10
Great Britain	*1,310* sps.						
Trees	73	7	5	—	78	4	6
Shrubs	136	1	7	15	63	—	14
Herbs	1,101	8	7	8	56	9	12
Italy	*3,079* sps.						
Trees	72	13	11	5	48	6	13
Shrubs	306	4	9	12	55	1	19
Herbs	2,701	5	5	13	56	11	10
China	*2,075* sps.						
Trees	501	14	10	3	56	—	17
Shrubs	1,574	10	6	11	62	—	11
Japan	*542* sps.						
Trees	174	12	14	2	60	—	11
Shrubs	347	7	7	13	66	—	7
Amazon Valley	*2 187* sps.						
Trees	941	3	.5	6	66	—	24
Shrubs	1,068	19	.5	6	65	—	9
Herbs	178	25	5	8	56	3	3
Madagascar	*539* sps.						
Woody Plants		11	1	4	73	—	11
Hawaii	*211* sps.						
Trees		8	1	4	76	—	11
Cape Colony	*380* sps.						
Trees		9	—	10	68	1	12
Natal	*303* sps.						
Trees	50	10	—	6	64	—	20
Shrubs	139	3	4	11	72	—	10
Herbs	114	6	13	6	66	8	1

modifications among herbs are often of very recent origin and that the important steps in the foliar evolution of the Dicotyledons took place while they were practically all woody in habit.

Evidence from various sources as to the relative antiquity of the six leaf types may be outlined as follows.

Palaeobotanical Evidence

Analyses of leaf type in various Cretaceous and Tertiary dicotyledonous floras have been made and are presented in the following table.[4] Owing to the frequent impossibility of determining whether an impression is that of a palmately or pinnately compound leaf, all the compound species have been thrown together. For simplicity's sake the few pinnate lobed forms have been included with the pinnate simple ones.

It will be noted that the percentage of compound leaves in the fossil floras is smaller than in the modern ones. This is probably due in part to the fact that compound leaves are usually broken up in fossilization into their component leaflets, which have often been described as complete leaves. The most striking difference, however, between ancient and present day floras is the much greater abundance in the former of palmate leaves (32 per cent as against 13 per cent in living species). In the Cretaceous the palmate-lobed forms were as numerous as the simple ones. In the Tertiary the lobed type was

[4] The following fossil floras have been examined:

Cretaceous: The Lower Cretaceous Deposits of Maryland, Berry (and others). Maryland Geological Survey; The Upper Cretaceous and Eocene Floras of South Carolina and Georgia, Berry. Professional Paper 84, U. S. G. S.; Flora of the Dakota Group, Lesquereux, Geol. Surv. of the Territories, Vol. VIII; Cretaceous Flora of the Territories, Lesquereux, Geol. Surv. of the Territories, Vol. VI; Flora of the Raritan Formation, Berry, Bull. 3, Geol. Surv. of New Jersey; Flora of the Amboy Clays, Newberry, Monograph XXVI, U. S. G. S.; Cretaceous Floras of Southern New York and New England, Hollick, Monograph L., U. S. G. S.; Flora Fossilis Arctica, Heer, including the Arctic, Patoot and Atane floras (Cretaceous) and those of the Polar Lands, Greenland, North Greenland, and Spitzbergen (Tertiary).

Tertiary. The Tertiary Flora of the Western Territories, Lesquereux, Geol. Surv. of the Territories, Vol. VII; The Flora of the Laramie Group, Ward, 6th Ann. Report, U. S. G. S.; The Flora of the Bad Lands, Lesquereux, Geol. Surv. of the Territories, Vol. VIII; Flore Fossile des Travertins Anciens de Sezanne, Saporta, Mem. Soc. Geol. de France (2), VIII; Tertiarfloren der Oesterr. Monarchie, Ettinghausen, Vienna, 1851; La Flore Fossile du Japon, Nathorst, Bih. Svensk. Vetensk-Akad. Handlingar, B. 20, No. 2.

TABLE II

	Species	Palm. Simple	Palm. Lobed	Pinnate	Compound
Cretaceous					
Potomac	22	6	7	6	3
S. Carol. and Georgia	68	11	3	46	8
Dakota	163	24	42	95	2
Territories	79	19	20	39	1
Raritan	114	12	14	75	13
Amboy Clays	106	15	16	67	8
S. N. Y. and N. Eng.	159	22	22	107	8
Arctic	32	3	1	20	8
Patoot	71	14	8	37	12
Atane	94	15	8	47	24
Total	908	141 (15.5%)	141 (15.5%)	539 (59%)	87 (10%)
Tertiary					
Territories	205	57	12	109	27
Laramie	130	46	8	68	8
Bad Lands	34	12	6	10	6
Polar Lands	84	24	3	48	9
Greenland	178	31	11	106	30
N. Greenland	46	13	4	24	5
Spitzbergen	50	17	5	26	2
Sezanne	70	19	3	40	8
Austria	31	3	4	18	6
Japan	49	7	5	30	7
Total	877	229 (26%)	61 (7%)	479 (55%)	106 (12%)
Total of fossil floras studied	1,785	370 (21%)	202 (11%)	1,018 (57%)	195 (11%)
Total woody plants in modern floras studied	7,014	661 (9%)	305 (4%)	4,578 (65%)	1,470 (21%)

little commoner than at present, but the palmate simple was much more abundant. Of course other fossil floras which are yet to be worked out, particularly those which are distinctly from tropical regions (where palmate forms are generally less numerous) may sometimes show conditions different from those which we have presented here; and fossil evidence in the present problem cannot be regarded as at all conclusive. The facts available at present, however, certainly seem to indicate that palmate leaves, particularly lobed ones, were much more abundant in earlier geologic time than at present.

MORPHOLOGICAL EVIDENCE

1. *The Node.*—Important evidence toward a solution of the present problem may be obtained from a study of the general topography of the node. One of the writers[5] has shown that among Dicotyledons

---

[5] The Anatomy of the Node as an Aid in the Classification of Angiosperms. Sinnott, E. W., Am. Jour. Botany, 1: 303–322. 1914.

the vascular supply to the leaf causes either a single gap (unilacunar type, fig. 85), three gaps (trilacunar type, figs. 83 and 84), or more than three gaps; that these types are very constant within large groups of plants, and that the trilacunar condition is probably the most ancient of all, the other two having been derived from it by amplification or reduction. It is pointed out that the main distinction lies really between the tri- and multilacunar, on the one hand (which in this paper we shall designate together as multilacunar), and the unilacunar on the other. That the former type is almost certainly more ancient than the latter is made evident by the fact that it characterizes the vast majority of all those orders (Amentiferae, Ranales, Rosales and Malvales) which are recognized as being relatively ancient and among which are presumably to be found the most primitive living Dicotyledons. The following table, based on the genera of Dicotyledons enumerated by Engler, also shows the distribution of these two nodal types among the trees, shrubs and herbs of the Archichlamydeae and Metachlamydeae.[6]

TABLE III

Genera.	Multilacunar.	Unilacunar.
Archichlamydeae		
Trees	972, 76%	909, 24%
Shrubs	794, 71%	322, 29%
Herbs	688, 59%	466, 41%
Metachlamydeae		
Trees	19, 6%	269, 94%
Shrubs	199, 20%	767, 80%
Herbs	752, 43%	994, 57%
Metachlamydeae, excl. Compositae		
Trees	4, 1%	269, 99%
Shrubs	26, 3%	767, 97%
Herbs	134, 10%	994, 90%

It is evident that the great majority of the Metachlamydeae (especially if we exclude the Compositae) are unilacunar and that the multilacunar forms are massed in the Archichlamydeae. The contrast is much more striking among woody plants (which are really the only ones significant in our problem) than among herbs. This predominance of the multilacunar node among the older orders and in the more primitive of the two great subclasses; and the confinement of the unilacunar type for the most part to less primitive groups, provide convincing evidence that the former condition is more ancient than the latter.

---

[6] Analyses of nodal type are based on a study of a very large number of species in about 700 genera, from practically all the families.

The importance of this conclusion is evident when we observe that there is a definite correlation between nodal topography and leaf type. This is indicated briefly for certain representative floras in the following table.

TABLE IV

	Palm. S.		Palm. L.		Palm. C.		Pinn. S.		Pinn. L.		Pinn. C.	
	Multi.	Uni.	Multi.	Uni.	Multi.	Uni.	Multi.	Uni.	Multi.	Uni.	Multi.	Uni.
	%	%	%	%	%	%	%	%	%	%	%	%
Tropical tree genera (Engler)	64	36	100	0	94	6	47	53	100	0	93	7
Temperate tree genera (Engler)	87	13	100	0	93	7	52	48	100	0	97	3
Northern United States												
Woody plants	92	8	100	0	97	3	65	35	100	0	86	14
Herbs	69	33	94	6	95	5	42	58	67	33	90	10
Great Britain												
Woody plants	100	0	100	0	100	0	77	23	100	0	91	9
Herbs	54	46	88	12	99	1	36	64	79	22	95	5
Italy.												
Woody plants	78	22	100	0	95	5	53	47	67	33	96	4
Herbs	54	46	94	6	97	3	38	62	78	22	92	8
China.												
Woody plants	79	21	97	3	93	7	56	44	—	—	91	9
Japan.												
Woody plants	84	16	96	4	97	3	59	41	—	—	94	6
Amazon Valley												
Woody plants	26[7]	74[7]	100	0	100	0	31	69	—	—	88	12
Herbs	66[7]	34[7]	83	17	93	7	30	70	67	33	100	0
Hawaii.												
Trees	94	6	100	0	100	0	35	65	—	—	100	0
South Africa.												
Trees (Sims)	90	10	100	0	84	16	34	66	—	—	100	0

It is apparent that among woody plants the great majority of palmate simple leaves are associated with a multilacunar node,[8] although this is not so evident among herbs. Practically all of the palmate lobed and palmate compound leaves, in both woody and herbaceous plants, belong to multilacunar species, and only a slightly smaller percentage of the pinnately compound leaves, as well. Fully half of the pinnate simple leaves, on the contrary, are connected with a unilacunar nodal type. In fact, the great majority of unilacunar plants have simple pinnate leaves. Of the 5,080 unilacunar species in the floras studied, 4,430, or 87 per cent, are characterized by foliage of this type. The pinnate-lobed type is too infrequent among woody plants to make figures derived from it of any significance. This correlation between venation and nodal topography is not surprising, since the single bundle of the unilacunar node would naturally tend to continue as a strong midrib (fig. 85), and the isolated traces of the multilacunar type would readily remain isolated and produce a palmate or compound lamina (fig. 84). In fact, the three lobes of a lobed leaf and the three leaflets of a trifoliately compound one are definitely related in their ontogeny to the point of origin of the three traces.

---

[7] Exclusive of Melastomaceae, Woody plants, 75—25; herbs, 80–20.

[8] Where the node is unilacunar with this foliar type, as among the Melastomaceae and certain of the Lauraceae, Verbenaceae and others, the leaf trace, although causing but a single gap, is almost always itself divided into three strands.

The definite association of the unilacunar type (which seems clearly not to be primitive) with the simple pinnate leaf certainly indicates that this leaf type, now so dominant among Angiosperms, is not at all the most ancient one. The many cases where a multilacunar node, also, is present with this foliar form are to be regarded on such a view as the persistence of an ancient character at the node when it has been lost elsewhere in the leaf.

2. *Floral Parts.*—The structure of certain supposedly conservative regions other than the node also furnishes evidence for the solution of our problem. One of these regions is the floral axis and its appendages. It is very noticeable that sepals, petals and floral bracts are often palmate in their venation when foliage leaves are otherwise. The homologies of the first two organs are still matters for debate, but bracts, at least, are almost certainly to be regarded as the morphological equivalents of leaves. A few cases of palmation in floral structures are shown in figures 25 to 36. Of course there are many instances of pinnate sepals, petals and bracts but these are very rare in families where the leaves are typically palmate; and palmate floral parts are very frequent in plants with pinnate leaves. The widespread occurrence of palmation in floral parts may be looked upon as the persistence of an ancient character which has often been lost elsewhere.

3. *The Seedling.*—The truth of the doctrine of recapitulation in its application to plants is frequently questioned at the present time but enough cases of conservatism in the seedling have been recorded to make evidence from this source worth seeking in any

such problem as the present one. A general survey of seed-leaf characters throughout the Dicotyledons, such as may conveniently be found in Lubbock's treatise on seedlings,[9] reveals the striking fact that the cotyledonary venation in the majority of cases is palmate, very often with three main veins. Not only are palmate cotyledons present in practically all palmate-leaved species but they occur (and often predominate) in every important pinnate-leaved family, as well. Some typical cases from all of the great Dicotyledonous orders are illustrated in figures 37 to 69.

The fact that the cotyledon is often broader in proportion to its length than is the vegetative leaf cannot explain its predominant palmate venation for it will be noted that in Acer, Antigonon, Rhus and many others the seed leaf is much narrower, proportionally, than the vegetative one but is nevertheless provided with three or more palmate veins. Nor can the palmate condition be explained as a physiological necessity for all seedlings, for there are numerous instances where the cotyledon is pinnate in venation. Taken in connection with the primitiveness of the trilacunar node, this prevalence of three-veined palmate cotyledons among Dicotyledons of all families must be regarded as important evidence that the palmate, three-veined leaf is indeed the most ancient type for the group. Should such a conclusion be clearly established, this preponderance of palmate venation in the embryo will evidently furnish an important instance of the persistence of an ancient character in plant ontogeny and will strongly support the theory of recapitulation in its application to the Angiosperms.

A somewhat similar phenomenon is provided by the peltate leaves of Tropaeolum (fig. 71), Hydrocotyle and other plants, where the early developmental stages are strongly palmate lobed (fig. 70). That this embryonic condition is ancestral for these species is indicated by its prevalence among closely related forms.

4. *Reversions.*—There are numerous cases among plants where very vigorous growth brings about a reversion to a primitive character or intensifies its ordinary development, and it might therefore be expected that large and vigorous leaves would tend to revert to a more ancient type. In most cases there is little difference in venation among the leaves of a single plant, but numerous exceptions do occur where the largest and rankest leaves are palmate lobed whereas the

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[9] A Contribution to our Knowledge of Seedlings, Lubbock, J.

smaller ones are simple palmate or pinnate. This fact is illustrated for Acer, Viburnum and Ampelopsis in figures 3, 5 and 6, 18 and 19, and 20 and 21. It has been observed by the writers in practically all families possessing palmately lobed leaves. There are also cases where a vigorous twig or stool shoot will produce this type of foliage although the typical leaf for the species is simple (figs. 72 and 73).

Evidence from morphology is therefore decidedly against the claims to primitiveness of the pinnate simple and pinnate lobed types, and as between the other four is clearly in favor of the greater antiquity of one of the palmate forms. Let us see whether such phylogenetic evidence as is available will permit a further narrowing down of the field.

PHYLOGENETIC EVIDENCE

Phylogenetic evidence first supports that from other sources in pointing to the simple pinnate leaf as more recent than the others, for it is overwhelmingly predominant in the Metachlamydeae. Of the 1,245 woody genera of this subclass enumerated by Engler 1,110 are simple pinnate, or 89 per cent; and of the 4,840 metachlamydeous species in the floras analyzed above, 3,780, or 78 per cent, are simple pinnate, and 400, or 8 per cent, are pinnate lobed, a total of 86 per cent. Of the 2,150 *woody* Metachlamydeae in these floras 1,915, or 89 per cent, have simple pinnate leaves. A type of leaf so closely associated with specialized floral structures is not likely to be very primitive. On the other hand, 70 per cent of the palmate simple type, 90 per cent of the palmate lobed, 93 per cent of the palmate compound and 88 per cent of the pinnate compound are included in the Archichlamydeae.

Of the four remaining types the pinnately compound, although developed for the most part among the Archichlamydeae, is found principally in the Juglandaceae, Rosaceae, Leguminosae, Zygophyllaceae, Sapindaceae, Rutaceae, Simarubaceae, Burseraceae, Meliaceae, Anacardiaceae, Araliaceae, Umbelliferae, Bignoniaceae and Oleaceae, none of which are generally regarded as being particularly ancient families. On phylogenetic evidence, therefore, the pinnately compound leaf cannot well be considered a primitive one.

To settle our problem we should be better acquainted than at present with the phylogeny of the Angiosperms. As to just what modern families should be regarded as closest to the ancient stock is as yet not definitely settled. All evidence now available, however, as to the structure of both vegetative and reproductive organs in the various families of the inclusive order Amentiferae seems to indicate clearly that this great group of simple-flowered plants is not ancient in type, as has frequently been supposed, but is rather to be looked upon as reduced and specialized. The prevalence of the simple pinnate leaf among its members is therefore no indication of the primitiveness of this foliar type. Those Dicotyledons which both from their wood structure and reproductive morphology are today winning recognition from phylogenists as being among the most ancient members of the whole class are included mainly among the simpler types of the Ranales, Rosales and Malvales. The very great frequency of the palmate leaf, either simple, lobed or compound, among the Ranunculaceae, Saxifragaceae, Hamamelidaceae, Malvaceae and their immediate allies (over half of the palmate lobed leaves in the floras investigated belong here) thus furnishes the most important evidence that modern phylogeny can contribute to a solution of the present problem and confirms the evidence from other sources that the palmate type is indeed the most ancient among Dicotyledons.

As to which of the three palmate forms is the oldest we cannot be quite sure. It is possible that the simple pinnate leaf with closed venation and the veins all converging at the tip of the lamina, a type which is predominant among Monocotyledons and the seed-leaves of Dicotyledons, may be the most ancient condition, from which the lobed form has arisen by a separation and divergence of the veins. The high percentage of palmate lobed leaves in the more ancient fossil floras, however; the primitiveness of the trilacunar node; the numerous cases of reversion of palmate simple to palmate lobed leaves, and the predominance of the palmate lobed form in the more primitive orders whereas the palmate simple and palmate compound types are often characteristic of more highly specialized groups, all point to the conclusion that a three-lobed, palmately three-veined leaf is one of at least very high antiquity. In such a leaf the three independent leaf trace bundles, widely separated at their insertion, probably remained so throughout their course into the lamina. In modern leaves of this type we usually find either three separate bundles in the petiole (fig. 81) or a single three-lobed one (fig. 80). Other leaf types generally possess a much more complicated petiolar system. If the primitive leaf was sessile or nearly so, as most writers agree, it is probable that the three traces went off pretty directly into the lamina as is shown in our reconstruction (fig. 83).

If the view thus set forth is a sound one we should be able to trace the transitional steps between such a primitive leaf and the various types of foliage which now occur throughout the Angiosperms.

Intermediate conditions between the palmate lobed and the palmate simple are to be found in several genera such as Acer, Rubus, Viburnum, Sterculia, Malus and others. In fact, the genus Acer (figs. 1 to 11) shows in its various species all transitions from simple pinnate to ternately compound. In many such plastic genera and families, of which the Araliaceae are another notable example, almost all leaf types may occur in closely related species and it is common to find several of them, with all sorts of intermediate conditions, even on the same plant. In all such cases vigorous growth emphasizes or restores the palmate lobed condition. In such groups as the Piperaceae, Melastomaceae and others the lobed condition seems to have been entirely lost, although the venation is still palmate. With the loss of the lobes the two lateral veins usually curve inward, sometimes even giving rise to a practically closed venation.

The palmate compound type has evidently arisen from the lobed one simply by an increase in the depth of the sinuses. The various steps may often be traced in the same species or even on one individual as in species of Acer (figs. 6 and 7), Rubus, the Araliaceae and many others. Since most palmately lobed leaves have three main veins, the compound ones derived from them are for the most part trifoliate. In many cases each leaflet of a trifoliate compound leaf becomes compound, in its turn, thus giving rise to the ternately compound type (fig. 11).

The pinnate simple leaf seems to have had its origin from the lobed type through the disappearance of the lobes (palmate simple) and a great reduction or disappearance of the lateral veins. Numberless transitional steps may be found, especially in the more plastic genera (figs. 1, 2 and 3, 12, 13 and 14). Most families which are preponderantly simple pinnate in leaf type have a few species or genera where the two basal veins are especially prominent or in which the leaf is essentially palmate. In certain cases (Rutaceae and others) the simple pinnate leaf probably represents the terminal leaflet of a reduced compound leaf.

The pinnate lobed type has apparently been derived from the pinnate simple by a more or less complete lateral dissection of the lamina, the lobes really representing very large leaf teeth. Transitions may be found in many herbaceous families (figs. 16 and 17).

The pinnate compound leaf evidently originated from the palmate compound, and transitions may frequently be observed in Acer (figs. 8 to 11), Rubus (figs. 14 and 15) and many other genera. In each case the two lowest leaflets are homologous with the lateral leaflets of the trifoliate type and the terminal leaflet has cut off basally one or more additional pairs. In practically all woody (and therefore more ancient) Dicotyledons the pinnately compound leaf seems to have arisen through this almost meristematic activity of the terminal leaflet. It is essentially a modified palmate leaf rather than a pinnate one, a fact which may explain what we have previously noted, its almost invariable correlation with a multilacunar node. In certain of the Proteaceae, however, and a great many herbaceous and semiherbaceous species leaves which are essentially pinnate compound have had their origin in an actual dissection of the lamina of a simple pinnate leaf.

In the even-pinnate Leguminosæ (and possibly in other families as well) the pinnately compound condition seems to have arisen from a palmately two-lobed rather than from a palmately three-lobed type. Transitions may be found from the simple palmate leaf of Cercis (fig. 22) through various species of Bauhinia (fig. 23) and related genera to forms with two distinct leaflets (fig. 24) which in still others have cut off pairs of leaflets basally to produce the typical even-pinnate type.

Factors in Foliar Evolution

The most important factor operative in altering the ancient three-lobed, three-veined palmate leaf seems to have been the origin of the petiole. It is generally agreed that this organ, the last part of the leaf to appear in ontogeny, is also more recent in evolutionary origin than the rest. It may well owe its development to the fact that the early Angiosperm leaf, ever increasing in breadth of lamina, was in need of a greater flexibility than was afforded by a broad sessile base in order to guard against havoc by winds and also, perhaps, to increase transpiration. The primitive leaf with its three traces widely separated in origin passing directly from node to lamina, was thus constricted at its base and its three bundles forced close together in the petiole. In some cases, as in a few leaves today (fig. 81), they stayed apart, though crowded close together, and diverged again at the base of the lamina (fig. 84). In others they partially fused by their margins (fig. 80). In most instances, however, they became joined together into a continuous cylinder (fig. 82) or were broken up into a ring of numerous strands. This petiolar system sometimes separated into approximately equal portions again at the base of the lamina but seems to have shown an increasing tendency to persist as a single strand, the midrib, sending off numerous branches on both sides. In other words, the midrib is the continuation of the petiole, to which it owes its origin. The approximation of the three traces in the petiole apparently extended down to the node and was responsible for the origin of the unilacunar nodal type which we have seen to be so intimately correlated with the simple pinnate leaf. Among woody plants the unilacunar type is largely confined to the tropics. This seems to be due to the fact that the tropical rain forest leaf is usually large and heavy and provided with a stout petiole, which for mechanical reasons is cylindrical and which thus has a circular leaf scar or point of attachment as opposed to the elongated scar of most temperate woody plants. This narrower point of insertion of course tends to pull the leaf traces more closely together and thus to develope the unilacunar node, which is also produced occasionally by the loss of the two lateral traces.

We are in almost complete ignorance of the other factors operative in the evolution of the leaf. The development of the palmate simple leaf from the palmate lobed one may well have been due, at least in the first instance, to reduction consequent upon loss of vigor or to decrease in size for other reasons. The acquirement of a leathery, xerophytic texture by the leaf is operative in some way to change the lobed leaf into a simple one, as is shown by the almost complete absence of palmate-lobed leaves among woody plants in the tropics (see Table I and tropical species of Rubus, figs. 12 and 13). Tropical leaves are often large and are frequently compound but the ultimate foliar unit, whatever it is, is generally narrow in shape and has a stout midrib. The reason for this is not clear, but it may be connected in some way with tropical xerophily.

As to what causes the development of compound leaves we are also uncertain. Such types, however, perhaps from their ability to orient themselves to sunlight, are well represented in warm, dry regions, as is shown by the abundance in such places of the Leguminosae, Proteaceae and other compound-leaved types. These families may well have acquired their leaf type under a more mesophytic environment (where most of the transitional conditions are found today) and thus have been especially fitted to invade arid regions.

Climatic Distribution of Foliar Types

The present distribution of leaf types with reference to climate provides us with evidence as to the climatic conditions under which Angiosperms first appeared. A study of Table I shows that the palmate-lobed leaf is conspicuously absent from the woody plants of tropical regions and is developed almost entirely in such forms under a temperate mesophytic environment, a fact of much suggestiveness since we have concluded that this leaf type is the most ancient of all. A number of families, such as the Rosaceae and Saxifragaceae, have numerous palmate species in the temperate zones but are represented in the tropics almost entirely by pinnate forms.

Palmately lobed leaves occur frequently among herbaceous plants in the tropics and, in fact, the distribution of the various leaf types among herbs is much the same under widely different climatic environments. This state of affairs, which we shall see duplicated in the distribution of nodal types in the various regions, is probably due to the fact that herbs, a recent and adaptable form of vegetation, live under very different circumstances from their more primitive woody relatives in that they can pass over adverse conditions underground or as seeds. Woody plants, because of their permanent aerial habit, are much more susceptible to climatic differences and have closer structural correlations with them. It was among such plants that the evolution of the various types of node and leaf undoubtedly took place.

We have brought forward evidence that the multilacunar type of node is primitive and definitely associated with the various forms of palmate leaves; and that the more recent unilacunar type is almost always connected with the simple pinnate leaf. The following table is therefore of interest in showing the distribution, among trees, shrubs and herbs, of the two nodal types in various regions of the globe.

It will be observed that among herbs the proportions of the two nodal types, like those of leaf shape, are not widely different in the tropics and the temperate regions, although unilacunar species are somewhat more common in the former than in the latter. Among shrubs, however, we may note a much higher percentage of multilacunar species in temperate areas than in arid or tropical ones, running from 70 per cent or 80 per cent in the former to 30 per cent or 40 per cent in the latter. Trees, however, which are probably the most ancient type of all, show the widest extremes and among them the relative proportion of the two nodal types is very closely correlated with climate. In the great land area of the north temperate zone

Table V

	Species	Trees		Shrubs		Herbs	
		Multi.	Uni.	Multi.	Uni.	Multi.	Uni.
		%	%	%	%	%	%
Northern United States	2,811	85	15	69	31	58	42
Rocky Mountains	1,405	90	10	84	16	62	38
Great Britain	1,231	94	6	81	19	56	44
Germany	1,120	97	3	81	19	54	46
Russian Empire	3,676	95	5	70	30	58	42
Alps	1,735	96	4	87	13	57	43
Spain	3,901	91	9	43	57	53	47
Italy	3,069	92	8	67	33	60	40
Flora Orientalis	9,848	71	29	59	41	63	37
Syria	2,570	84	16	54	46	68	32
Egypt	1,138	83	17	53	47	63	37
China	2,075	76	24	67	33		
Japan	542	87	13	63	37		
Patagonia	1,405	84	16	66	34	67	33
Chili	2,160	76	24	67	33	67	33
New Zealand	974	72	28	47	53	61	39
Hongkong	681	69	31	41	59	52	48
Simla	987	76	24	61	39	56	44
Gangetic Plain	996	60	40	60	40	54	46
Florida Keys	466	56	44	50	50	47	53
Australia (extratropical)	5,874	51	49	67	33	63	37
South Africa	7,284	60	40	58	42	45	55
Amazon Valley	2,174	57	43	30	70	38	62
British West Indies	2,191	46	54	46	54	50	50
Nicaragua	1,496	66	34	54	46	58	42
Tropical Africa	10,520	54	46	32	68	39	61
Mauritius	555	45	55	42	58	56	44
Ceylon	1,752	50	50	43	57	36	64
Bombay, Uplands	996	61	39	50	50	50	50
Bombay, Lowlands	1,229	50	50	48	52	46	54
Dutch East Indies	6,246	49	51	40	60	44	56
Malay Peninsula	3,049	49	51	32	68	26	74
Manila	332	66	34	47	53	44	56
Hawaii	508	50	50	41	59	51	49

from 85 per cent to 95 per cent (averaging 90 per cent or more) of the arborescent species have three or more gaps at the node. This type is also dominant in the temperate non-arid regions of the Southern Hemisphere, such as Chili, Patagonia and New Zealand; and, to a less extent, in upland areas in or near the tropics, as Nicaragua, Simla and the Bombay uplands. In the tropics, however, there is a strong contrast for here 50 per cent or more of the tree species are unilacunar. Almost all the unilacunar arborescent genera are typically tropical in their distribution. Subtropical regions, such as the Florida Keys, the Gangetic Plain and Hongkong, show a percentage intermediate between the two extremes. In South Africa and extra-tropical Australia, the great arid areas of the South Temperate zone, the unilacunar type is only slightly less common than in the tropics. In connection with this concentration of the presumably primitive trilacunar woody type in generally temperate and mesophytic habitats, it should also be borne in mind that most of the genera and families regarded as being particularly ancient are best developed in temperate or warm-temperate regions.

These facts, which are in agreement with a large body of evidence gathered by the writers from other sources, all point strongly to the conclusion that tropical plants are specialized in almost every particular and that the Angiosperms therefore could not have arisen under a tropical environment, as has generally been taken for granted, but must have had their origin in a climate which, though doubtless very equable and devoid of extremes of temperature, was essentially a temperate one. Such a climate, as far as we are able to judge of conditions in the Mesozoic, could only obtain, as a general rule, in upland or mountainous regions. On such a supposition the Mesozoic Angiosperms which we know are to be regarded as plants which had migrated down into the tropical lowlands and swampy areas, where they could readily be preserved as fossils. If the earliest Dicotyledons were indeed largely confined to temperate upland regions (where fossilization could almost never take place), does not this explain why we know of so very few Mesozoic Angiosperms and why these are so diverse and often so highly specialized and far from primitive? And does it not strongly suggest the possibility that on these ancient mountains, about the vegetation of which we know so little, Angiosperms may have been developing in extremely remote times, considerably earlier than the Lower Cretaceous?

Phylogenetic Conclusions

If the hypothesis which has here been presented is a sound one and the trilacunar palmate leaf type is indeed the most ancient among Angiosperms, the theory which derives these dominant seed plants from Cycad-like Gymnosperms meets with grave difficulty. The Mesozoic Bennettitales, from which many phylogenists believe the Angiosperms to have descended, were unilacunar and possessed, like all Cycads, pinnately compound leaves. The difficulty of deriving from such a leaf the foliar types which prevail among living Angiosperms has been noted by many writers. Wieland[10] suggests that the gap may have been bridged by a fusion of the leaflets of the compound leaf, with the eventual production of a simple pinnate one. But if a palmate leaf was indeed the primitive Angiosperm condition it is difficult to see how it could have arisen from any cycadaceous type. The Conifers, on the other hand, are invariably palmate in their venation. Although the common leaf type is a narrow one with but one or two strands, there are numerous cases in the Araucarineae and Podocarpineae where the leaf becomes broader. In accommodation to this change the vascular tissue increases in amount, but always by a basal multiplication of the strands in palmate fashion rather than by the origin of branches from a midrib (figs. 74 and 75). It is possible to imagine how such a broad-leaved form, developing a trilacunar type of insertion (Agathis is occasionally bilacunar) might easily give rise to the palmate leaf which we have regarded as ancient. Evidence from the leaf certainly favors a coniferous rather than a cycadean stock as ancestral for the Angiosperms.

Information from this source is also important in connection with the ancestry of the Monocotyledons. These plants are almost invariably palmate in their venation, and in many cases are three-veined. In the broad-leaved members of the Potamogetonaceae, Alismaceae, Araceae, Liliaceae, Dioscoreaceae and others we often meet with leaves which are essentially like those of the Piperaceae, Melastomaceae and other palmate Dicotyledons (figs. 76 and 77). The nodal conditions in such forms often suggest the dicotyledonous type, three traces departing to each leaf (fig. 78), and the petiole is also frequently trifasciculate. The cotyledon and early leaves in several families of Monocotyledons have been shown by Chrysler[11] and Coulter and Land[12] to have three bundles (fig. 79), apparently a vestige of the more ancient condition. The conspicuous tendency for the cotyledonary veins in the seed-leaves of many Dicotyledons to unite at the tip of the lamina (figs. 21, 27, 28, 38, and 44) producing a closed venation like that of the Monocotyledons, suggests either that the Monocotyledons are the more ancient of the two classes, a conclusion opposed by too much evidence from other sources; or that the leaf in these plants has been developed by a process of reduction much like that gone through by the cotyledons of Dicotyledons and by the mature leaves of certain members of that class. The Monocotyledons apparently had their origin from a palmate dicotyledonous stock, very likely at an early period when the palmate leaf was predominant among all Angiosperms.

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[10] Was the Pterophyllum Foliage Transformed into the Leafy Blades of Dicotyls? Wieland, G. R., Am. Jour. Science 38: 451–460. 1914.

SUMMARY

1. The primitive Angiosperm leaf was palmate in type, probably lobed, and was provided with three main bundles which arose separately at the node.

2. This conclusion is based on evidence from palaeobotany, that the palmate leaf was more frequent in the Cretaceous and Tertiary than at present; from morphology, that there is a correlation between the palmate leaf and the multilacunar node and between the pinnate leaf and the unilacunar node and that the former nodal type is the more ancient, that cotyledons and floral leaves are much more frequently palmate than are vegetative ones, and that vigorous growth emphasizes the palmate type; and from phylogeny, that palmate leaves are most frequent in relatively primitive groups and pinnate leaves in more advanced ones.

3. Transitions from the palmate lobed type to all other leaf forms may readily be traced.

4. The chief factor in the evolution of the now dominant pinnate leaf seems to have been the development of the petiole, in which

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[11] The Development of the Central Cylinder of the Araceae and Liliaceae, Chrysler, M. A., Bot. Gaz. 38: 161–184. 1904.

[12] The Origin of Monocotyledony, Coulter, J. M. and Land, W. J. G., Bot. Gaz. 57: 509–519. 1914.

the three originally distinct leaf bundles were pulled together into a single strand, the midrib.

5. Among woody plants, the multilacunar (more ancient) nodal type predominates in temperate regions and the unilacunar (more recent) in the tropics. The palmate lobed leaf among such plants is also almost entirely confined to temperate regions. These facts, in company with others, indicate that the Angiosperms first appeared under a climate more temperate than tropical, a climate in the Mesozoic probably found only in the uplands. It is suggested that the absence of the earliest Angiosperms as fossils may be due to their confinement to such upland regions, where preservation would be very difficult.

6. Evidence from the leaf strongly favors the view that the Angiosperms sprang from a coniferous (palmate) rather than from a cycadean (pinnate) stock.

7. The Monocotyledons were derived from some ancient palmate group of Dicotyledons.

The writers are much indebted to the authorities of the Gray Herbarium and of the Arnold Arboretum for the use of their libraries and herbaria.

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DESCRIPTION OF FIGURES OF PLATES I–IV

Fig. 1. *Acer carpinifolium* Sieb. & Zucc. (pinnate simple).

Fig. 2. *Acer tataricum* L. (palmate simple).

Fig. 3. *Acer ginnale* Maxim., vigorous leaf (palmate lobed).

Figs. 4 and 5. *Acer ginnale* Maxim. leaves of less vigorous growth.

Figs. 6 and 7. *Acer glabrum* Torr., two leaves from the same twig showing origin of palmately compound type.

Figs. 8, 9, 10 and 11. *Acer Negundo* L., series of leaves showing transitions from trifoliate to ternately compound.

Figs. 12 and 13. *Rubus negroensis* Elm. and *Rubus benguitensis* Elm., tropical species reduced from primitive type.

Fig. 14. *Rubus odoratus* L., presumably primitive type for the genus.

Fig. 15. *Rubus neglectus* Peck, leaf showing the transition from palmately to pinnately compound.

Figs. 16 and 17. *Solanum nigrum* L. and *Solanum carolinense* L. two closely related species showing transition from pinnate simple to pinnate lobed.

Figs. 18 and 19. *Ampelopsis tricuspidata* Sieb. & Zucc., a leaf of vigorous and one of weaker growth.

FIGS. 20 AND 21. *Viburnum acerifolium* L., a leaf of vigorous and one of weaker growth.

FIGS. 22, 23 AND 24. *Cercis occidentalis* Torr., *Bauhinia* sp. and *Hymenostegia* sp., series showing origin of the pinnately compound type among certain of the Leguminosae.

FIG. 25. *Engelhardtia spicata* Blume, floral bract (leaf pinnate compound).

FIG. 26. *Betula nigra* L., floral bract (leaf pinnate simple).

FIG. 27. *Carpinus caroliniana* Walt., floral bract (leaf pinnate simple).

FIG. 28. *Bocconia frutescens* L., floral bract (leaf pinnate lobed).

FIG. 29. *Dalechampia Passiflora* C. &. H., floral bract (leaf palmate simple).

FIG. 30. *Adhatodia vasica* Nees, floral bract (leaf pinnate simple).

FIG. 31. *Berberis Bealei* Curt., petal (leaf pinnate simple).

FIG. 32. *Schizophragma hydrangeoides* Sieb. & Zucc., petal (leaf pinnate simple).

FIG. 33. *Guichenotia macrantha* Turcz., petal (leaf pinnate simple).

FIG. 34. *Anisoptera glabra* Kurz, calyx lobe (leaf pinnate simple).

FIG. 35. *Calycopteris floribunda* L., calyx lobe (leaf pinnate simple).

FIG. 36. *Mussaenda tenuiflora* Benth., petal (leaf pinnate simple).

FIG. 37. *Casuarina stricta* Ait., cotyledon (leaf greatly reduced).

FIG. 38. *Pterocarya fraxinifolia* Spach, cotyledon (leaf pinnate compound)

FIG. 39. *Betula nigra* L., cotyledon (leaf pinnate simple).

FIG. 40. *Celtis occidentalis* L., cotyledon (leaf palmate simple).

FIG. 41. *Grevillea robusta* A. Cunn., cotyledon (leaf pinnate compound).

FIG. 42. *Antigonon leptopus* Hook., cotyledon (leaf pinnate simple).

FIG. 43. *Mirabilis jalapa* L., cotyledon (leaf pinnate simple).

FIG. 44. *Rivinia aurantiaca* Warsz., cotyledon (leaf pinnate simple).

FIG. 45. *Delphinium formosum* Boiss. & Hook., cotyledon (leaf palmate lobed).

FIG. 46. *Brassica olearacea* L., cotyledon (leaf pinnate simple).

FIG. 47. *Crataegus oxyacantha* L., cotyledon, after Lubbock (leaf pinnate simple).

FIG. 48. *Mimosa pudica* Mill., cotyledon (leaf pinnate compound).

FIG. 49. *Canarium strictum* Roxb., cotyledon, after Lubbock (leaf pinnate compound).

FIG. 50. *Euphorbia pulcherrima* Willd., cotyledon (leaf pinnate simple).

FIG. 51. *Rhus typhina* L., cotyledon (leaf pinnate compound).

FIG. 52. *Acer saccharum* Marsh, cotyledon (leaf palmate lobed).

FIG. 53. *Rhamnus cathartica* L., cotyledon (leaf pinnate simple).

FIG. 54. *Ampelopsis tricuspidata* Sieb. & Zucc., cotyledon (leaf palmate lobed).

FIG. 55. *Elaeocarpus oblongus* Smith, cotyledon, after Lubbock (leaf pinnate simple).

FIG. 56. *Tilia americana* L., cotyledon (leaf palmate simple).

FIG. 57. *Althaea rosea* Cav., cotyledon (leaf palmate lobed).

FIG. 58. *Passiflora alata* Ait., cotyledon (leaf pinnate simple).

FIG. 59. *Lecythis Ollaria* L., cotyledon, after Lubbock (leaf pinnate simple).

FIG. 60. *Aucuba japonica* Thunb., cotyledon, after Lubbock (leaf pinnate simple).

FIG. 61. *Eucalyptus calophylla* R. Br., cotyledon (leaf pinnate simple).

FIG. 62. *Argania sideroxylon* Roem. & Schult., cotyledon (leaf pinnate simple).

FIG. 63. *Diospyros Lotus* L., cotyledon (leaf pinnate simple).

FIG. 64. *Ipomaea purpurea* Roth, cotyledon (leaf palmate simple).

FIG. 65. *Eranthemum leuconeurum* Fisch., cotyledon, after Lubbock (leaf pinnate simple).

FIG. 66. *Coffea arabica* L., cotyledon, after Lubbock (leaf pinnate simple).

FIG. 67. *Viburnum Opulus* L., cotyledon (leaf palmate-lobed).

FIG. 68. *Cucumis Melo* L., cotyledon (leaf palmate-lobed).

FIG. 69. *Senecio multiflorus* DC., cotyledon (leaf pinnate simple).

FIG. 70. *Tropaeolum majus* L., young leaf, near the growing point.

FIG. 71. *Tropaeolum majus* L., mature leaf.

FIG. 72. *Pterospermum Heyneanum* Wall., leaf from coppice shoot.

FIG. 73. *Pterospermum Heyneanum* Wall., leaf from mature branch (both after Brandis).

FIG. 74. *Podocarpus Nagi* Pilger, many-veined (palmate) leaf.

FIG. 75. *Podocarpus Hallii* Kirk, primitive type of leaf, with single bundle.

FIG. 76. *Sagittaria latifolia* Willd., mature leaf.

FIG. 77. *Trillium cernuum* L., mature leaf. Both this and the last are essentially like many dicotyledonous leaves in venation.

FIG. 78. *Trillium cernuum* L., diagrammatic cross section through the node, showing the similarity to the dicotyledonous type.

FIG. 79. *Agapanthus umbellatus* L'Her., diagrammatic cross section through the seedling, showing the three bundles in the cotyledon and in the first leaf (after Coulter and Land).

FIG. 80. *Viburnum acerifolium* L., cross section through the petiole.

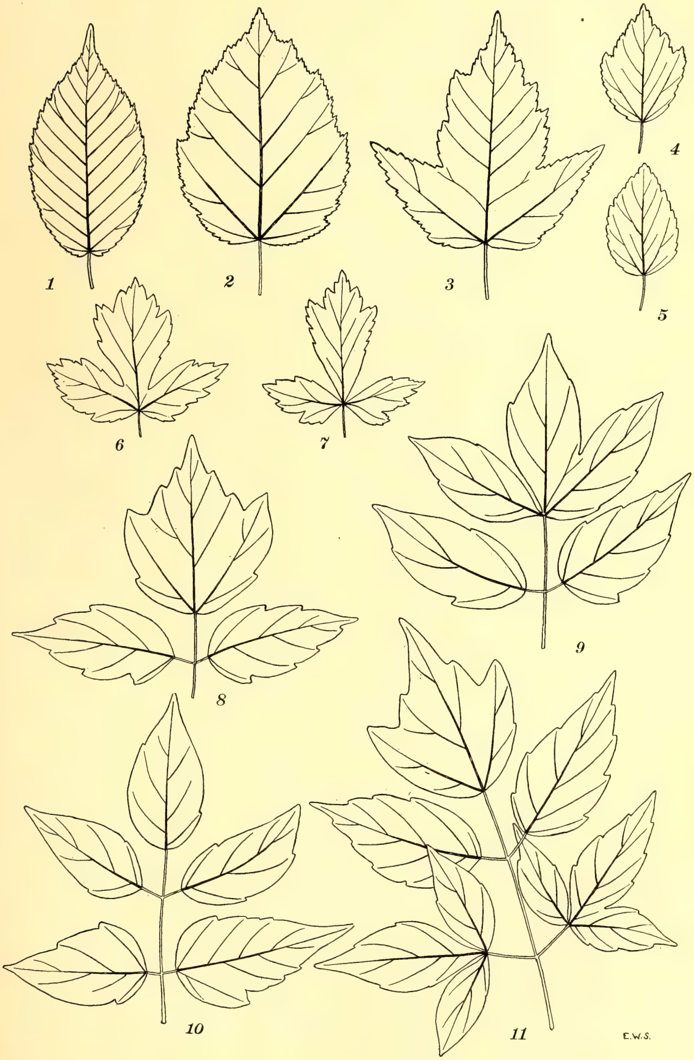
FIG. 81. *Ribes rubrum* L., cross section through the petiole.

FIG. 82. *Ulmus americana* L., cross section through the petiole.

FIG. 83. Reconstruction of the leaf (with cross section of the node) supposedly primitive for the Angiosperms. The node is trilacunar and the veins depart directly into the lamina without approximation in a petiole.

FIG. 84. A typical modern palmate lobed leaf. The node is trilacunar but the traces are pulled closely together in a narrow petiole before separating again in the lamina.

FIG. 85. A pinnate simple leaf. The node has become unilacunar and the single trace continues as a strong midrib.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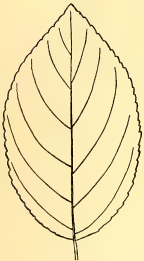
SINNOTT AND BAILEY: FOLIAR EVIDENCE AS TO ANCESTRY OF ANGIOSPERMS.



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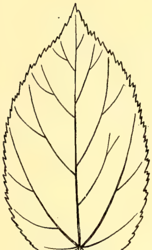

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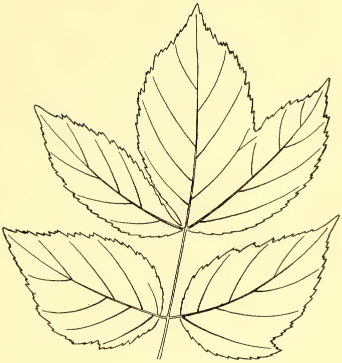

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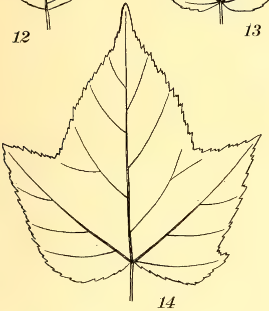

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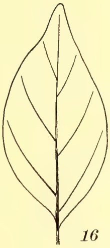

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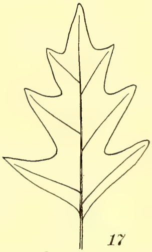

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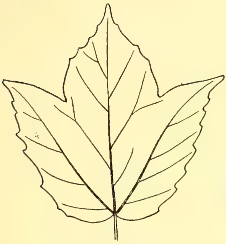

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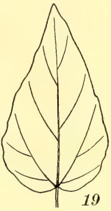

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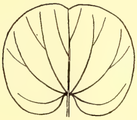

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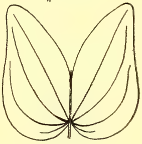

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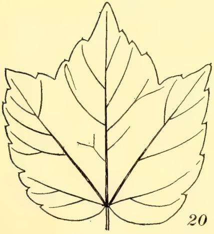

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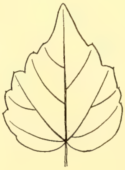

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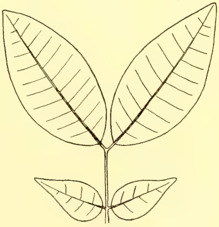

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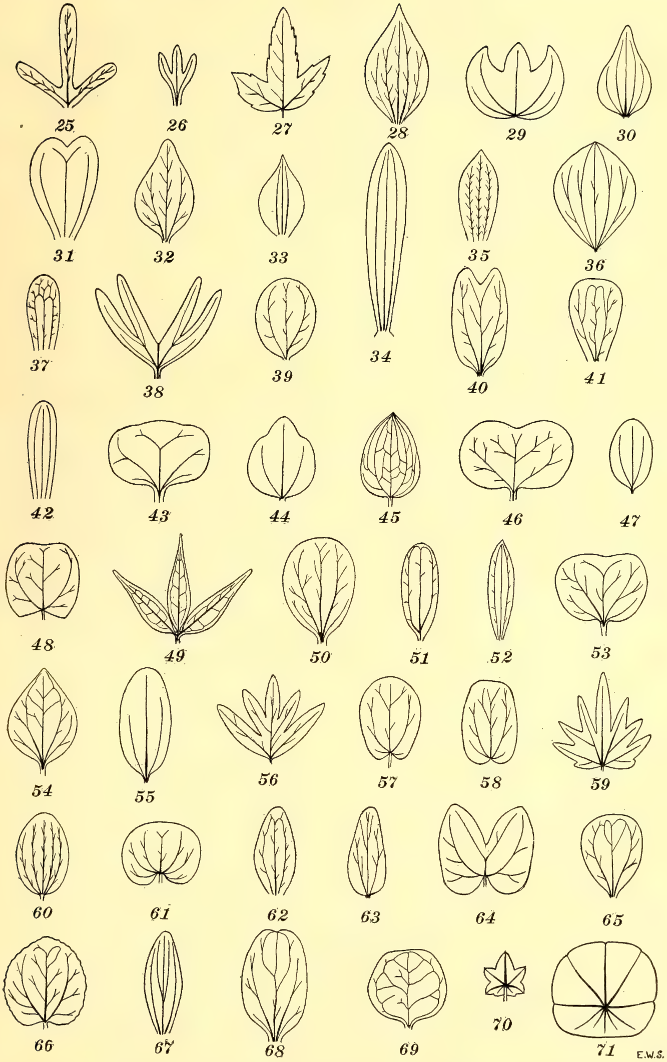
SINNOTT AND BAILEY: FOLIAR EVIDENCE AS TO ANCESTRY OF ANGIOSPERMS.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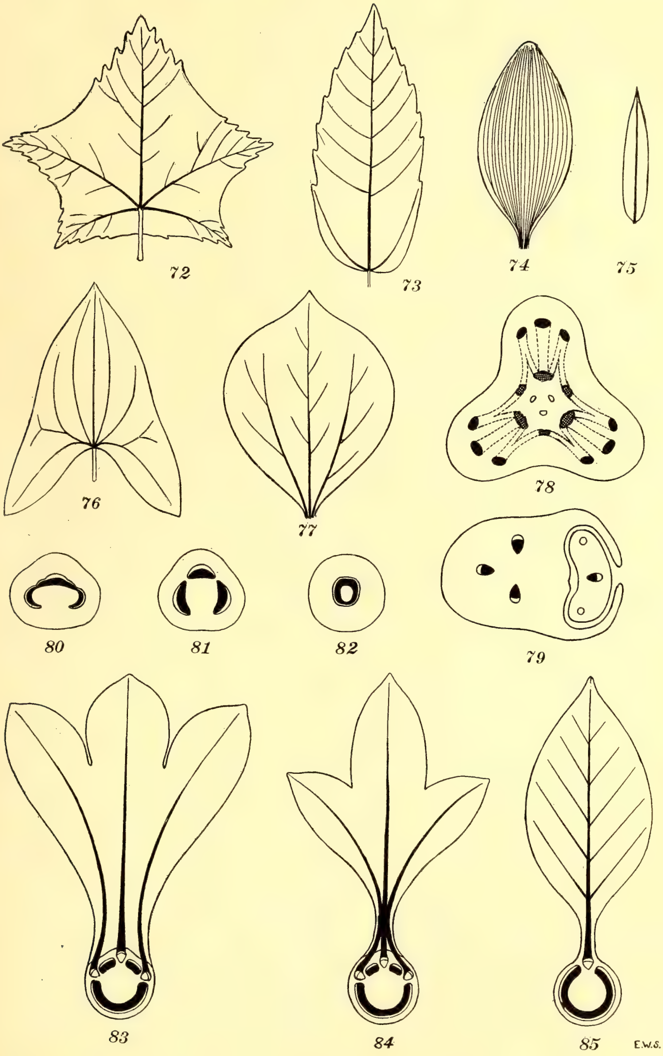
SINNOTT AND BAILEY: FOLIAR EVIDENCE AS TO ANCESTRY OF ANGIOSPERMS.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Sinnott and Bailey: Foliar evidence as to ancestry of Angiosperms.

THE GROWTH-FORMS OF THE FLORA OF NEW YORK AND VICINITY[1]

NORMAN TAYLOR

There has recently appeared in a work on the flora of New York,[2] an account of the relation between climate and the vegetation in which the length of the growing season was used as the chief temperature factor. This was done for the reason that it seemed to account for the distribution of the flora more closely than any other ascertainable temperature factor. While it is difficult to conceive of any climatic agency, such as the number of frostless days, the maximum or minimum temperatures, or the accumulated heat units, as actually controlling the distribution of the flora, yet it is a matter of common observation that temperature does affect vegetation and its distribution. How, then, are we to measure the effect of climate, and particularly temperature, on plant life? All of the older methods, including the one used in the flora of the vicinity of New York, have studied climate as a rather distinct entity, and then imposed a somewhat rigid, usually instrumentally correct scheme, on a complex aggregate like a local flora. All such schemes require considerable wrenching of the purely climatic factors on the one hand, as they certainly do of the assumed vegetative response on the other. Until recently, and with the possible exception of Merriam's "Life Zones," all studies of the effect of temperature on plants were of this type. They were essentially attempts to explain the facts of plant distribution by measured temperatures or heat units or frostless days or by some combination of these methods.

Raunkiaer[3] has studied temperature factors from an entirely different viewpoint. His idea, briefly, is that we must study climate, not as such, but as it is reflected in the vegetation which we all know it to have controlled. Such a method is not at all in line with older studies, it is really an *ex post facto* method of determining climate by the character of the vegetative response. From the standpoint of the ecologist and plant geographer, what could be more logical and reasonable? Temperature factors are not what one makes them out to be with an elaborate instrumental or mathematical method; they are rather what one finds them as reflected in the vegetation itself. Of course, in order to get the quality and kind of this implied vegetative response, we must study plants in a slightly new light, and Raunkiaer has devised, after Warming and a few earlier writers, a scheme for such a study.

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[1] Brooklyn Botanic Garden Contributions, No. 9.

[2] Taylor, N. Flora of the vicinity of New York: A contribution to plant geography. Mem. N. Y. Bot. Garden 5: 1–683. 1915. See pp. 33–36. All the statistics and figures used in this paper may be verified in that book. The nomenclature of the present paper is essentially that of the Gray's Manual.

[3] A fairly complete account of Raunkiaer's system, with a bibliography, may be found in Journ. Ecol. **1**: 16–26. 1913.

His theory is that plants react to climate by the kind and amount of protection exhibited by the perennating growth points during the winter or critical season. Upon this assumption he divides all vegetation into several different groups of growth-forms sometimes called life-forms, depending on the kind and amount of protection to their growing buds exhibited by each. In the following account, I have included only those of his growth-forms that are found in eastern North America, which may be characterized as follows:

Phanerophytes. Woody plants of all types, both evergreen and deciduous and exhibiting the least amount of protection from the cold, as showing the greatest amount of exposure. The group may be divided into **Megaphanerophytes,** trees over 30 m.; **Mesophanerophytes,** trees 8–30 m.; **Microphanerophytes,** shrubs or trees 2–8 m.; **Nanophanerophytes.** shrubs under 2 m. Examples of all these are too common to need citing.

Chamaephytes. Perennial by virtue of the fact that the buds are just above the ground, or on the surface, and are thus often protected by the snow blanket. Among local species *Arctostaphylos Uva-Ursi*, *Epigaea*, *Convolvulus*, etc., are good examples. It includes, also, cushion-plants.

Hemicryptophytes. With dormant buds in the upper crust of the soil, the top of the plant dying down in the winter. Common examples suggest themselves, as all our shallow-rooted herbaceous perennials belong here.

Geophytes. Perennial by bulbs, rhizomes, tubers or by root-buds. Examples among our native plants: most Orchidaceae, Liliaceae, *Sanguinaria*, *Hydrastis*, etc.

Helophytes and Hydrophytes. The former has buds at the bottom of the water. They are mostly marsh species such as *Typha*, *Sparganium*, *Acorus*, etc. Hydrophytes have perennating rhizomes or winter-buds and are truly aquatic, such as *Castalia*, *Elodea* and *Potamogeton*.

Therophytes. Annuals.

A tabular view of these different growth forms, with the abbreviations as used in this paper follows:

MG = Megaphanerophytes
MS = Mesaphanerophytes
MC = Microphanerophytes
N = Nanophanerophytes
CH = Chamaephytes
H = Hemicryptophytes
G = Geophytes
HH = Helophytes and Hydrophytes
T = Therophytes

Raunkiaer's method of adapting the study of these growth-forms as related to temperature is to estimate the number of species characterized by these different forms, and to get the percentages of the different growth-forms in the flora. For the purpose of comparison he established a "normal spectrum" which is constructed on purely hypothetical lines. It consists of 400 species carefully chosen from 1,000 representative species. The analysis of these 400 species into their different growth-forms gives, theoretically, the ideal phytoclimatic spectrum of the whole earth. According to Raunkiaer the percentage of species belonging to each growth-form, in the ideal spectrum of 400 species, is as follows:

PERCENTAGE OF GROWTH-FORMS IN NORMAL SPECTRUM[4]

Type of growth-form	MG & MS	MC	N	CH	H	G	HH	T
Percentage of growth-forms	6	17	20	9	27	3	1	13

These percentages are supposed to reflect the average condition as to the growth-forms of the whole earth. Of course they may need future revision; it would be strange if they did not as our knowledge of the habits of various species increases.

The method of comparing the climate of different parts of the earth's surface, on this conception, involves working out the percentages of the different growth-forms exhibited in the areas and a comparison of the figures thus obtained. The following table gives a few of the percentages as determined by Raunkiaer for widely separated areas. The percentages of the normal spectrum are given for comparison.

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[4] I have omitted epiphytes and stem-succulents, as being two of Raunkiaer's groups hardly applicable to our area.

Percentages of Growth Forms in Biological Spectra, after Raunkaier

Type of Growth-form	MG and MS	MC	N	CH	H	G	HH	T
Normal spectrum	6	17	20	9	27	3	1	13
Baffin's Land			1	30	51	13	3	2
Georgia	5	7	11	4	55	4	6	8
Denmark	1	3	3	3	50	11	11	18
Seychelles	10	23	24	6	12	3	2	16
Libyan Desert		3	9	21	20	4	1	42

These figures give some idea of the variation of climate implied by the different growth-forms predominating in the different areas. They also make more cogent the terms phanerophytic, hemicryptophytic and chamaephytic as applied to climate.

Raunkiaer has indicated three types of climate, as shown by his study of growth-forms, namely a tropical area, an area of decreasing warmth correlated with an increasing difference between winter and summer temperatures and with a favorable distribution of precipitation, and lastly a region with decidedly decreasing temperature, or also, very commonly, with an unfavorable distribution of precipitation, such as in deserts. The tropics are typical of the first of these conditions, the eastern sides of North America and Asia are typical of the second, and the arctic region and some deserts are characteristic of the third type of climate, which may be seen also in our own Southwest. Many refinements of these rather gross outlines of climate have been worked out, based on the so-called biochore, which is a line with the same percentages of a definite growth-form, as found in different parts of the continent. Such a study of the flora of North America would be extremely interesting. It can be rightly based only on complete percentages of growth-forms for different parts of the country, and it is with the idea of supplying this for the local flora area that the present paper has been written.

In attempting to apply Raunkiaer's principles described above and to get the biological spectrum of the flora near New York, I have thrown out of the calculation all the 615 species of introduced weeds, the 85 ferns and their allies, and 24 parasites. This leaves 1,907 native species that are found, roughly speaking, within 100 miles of New York City. Each species has been put in one or other of the categories mentioned above with the following result.

Biological Spectrum of the Flora of New York and Vicinity[5]

Growth-form	MG	MS	MC	N	CH	H	G	HH	T
Gymnosperms......		15	2	2					
Monocotyledons....			5		53	197	202	164	57
Dicotyledons.......	10	62	130	65	48	438	195	60	191
Totals...........	10	77	137	67	101	635	397	224	248
Percentages of growth-forms.....	% .52	% 4.03	% 7.18	% 3.51	% 5.29	% 33.29	% 20.23	% 11.74	% 13

The most remarkable figure in this list is the high percentage of geophytes, 20.23 per cent. For no region in the world has there been published such a large percentage of these plants with bulbs, rhizomes, corms and other subterranean methods of winter protection. Among the 692 native monocotyledons in the area, over 29 per cent are geophytes, while for 1,200 native dicotyledons there are only 16 per cent of the same growth-form. Undoubtedly the high percentage of monocotyledons in our flora, the pine-barrens of New Jersey are especially rich in them, has much to do with the large percentage of geophytes. Most of the regions with high geophyte percentages are arctic or sub-arctic; and, from this point of view, the high geophyte percentage in our area is misleading and it may be a response to quite other factors than climatic ones. As compared to the percentages of the normal spectrum those for the local flora are higher in the case of the aquatics, geophytes, and hemicryptophytes, lower in the case of chamaephytes and all the phanerophytes, and the same in the percentage of annuals.

Figures for other countries, mostly northern, show for hemicryptophytes averages ranging from 50 per cent to 60 per cent, the local flora percentage is only 33.29 per cent, well illustrating the condition that prevails in our area, where only a moderately large number of species are of northern origin. The percentage of all phanerophytes in our area is about 14.88, in the normal spectrum it is 43 per cent, in the Seychelles it is 57 per cent. Upon Raunkiaer's assumption that phanerophytes are typical of warm and tropical regions the figures of the local flora are somewhere near what one would expect.

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[5] Not counting .57 per cent of stem-succulents.

In the flora of New York and vicinity 13 per cent of the wild species are southern plants reaching their northern distribution outposts in the area within 100 miles of the city. We should expect to find these southern plants exhibiting a greater percentage of growth-forms characteristic of the warmer parts of the earth, than of those characteristic of the north. In this same area, also, 8 per cent of the native plants are typically northern and reach their southern distribution outposts in the region within about one hundred miles of the city. We should expect to find the percentages of growth-forms characteristic of the north predominating in these northern species. The following table gives the percentages of growth-forms in the southern species, the northern species, and, for comparison, the percentages in the whole native flora. The normal spectrum is included again, for comparison.

PERCENTAGES OF GROWTH-FORMS IN THE NORTHERN AND SOUTHERN SPECIES OF THE FLORA OF N. Y. AND VICINITY THAT REACH THEIR DISTRIBUTION OUTPOSTS IN THE AREA

	MG	MS	MC	N	CH	H	G	HH	T
Normal spectrum...	6		17	20	9	27	3	1	13
Whole native flora..	.52	4.03	7.18	3.51	5.29	33.29	20.23	11.74	13
Southern species....		5.62	7.08		7.83	30.97	20.53	7.83	14.92
Northern species....		1.31	3.94	8.55	8.55	26.31	24.34	23	3.94

There are, of course, hundreds of northern and southern species that range north or south of the local flora area,[6] but it seemed best to ignore these, and consider only those that find either their southerly or northerly distribution outposts within the region.

An analysis of these percentages of northern and southern species shows that the main lines of Raunkiaer's scheme are admirably illustrated in the local flora. We find in the southern species about 12 per cent shrubs or trees, in the northern species only 5 per cent, well illustrating the tendency for the woody plants to increase in size and profusion as we travel southward. Among the small undershrubs there appear to be none in the southern group, but over 8 per cent in the northern, which is considerably over the percentage for this growth-form in the whole flora. One of the most notable elements is the large percentage of HH in the northern species (23 per cent) and the large percentage of geophytes in the same group (24 per cent). Both these figures are so much above those published for any other region, that they may be open to the suspicion that other than climatic factors have influenced them. This region in eastern North America has never had this particular criterion of temperature response applied to it, in fact I know of no region where such a large number of species as our 1907 native plants have been used in making up the percentages as published by Raunkiaer, Vahl, and Paulsen for other countries. On the basis of the large numbers of species considered, and the obviously temperate nature of our climate, it may be that the normal spectrum as now understood is in need of revision. Certainly it seems to be much too low in the case of geophytes and the aquatics, and too high in the microphanerophytes.

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[6] The area included is as follows: All of the State of Connecticut; in New York the counties bordering the Hudson River up to and including Columbia and Greene also Sullivan and Delaware counties, and all of Long Island; all of New Jersey; and in Pennsylvania, Pike, Wayne, Monroe, Lackawanna, Luzerne, Northampton, Lehigh, Carbon, Berks, Bucks, Schuylkill, Montgomery, Philadelphia, Delaware and Chester counties.

There are 19 northern species in the area, reaching their southerly distribution outposts here, but found only at elevations in excess of 1,000 ft., and most closely approximating an alpine habitat of any of our native plants. Of course, percentages of growth-forms based on such a small number of species are apt to be misleading, but as illustrating a tendency they prove interesting. The following is a list of such species:

Growth-forms of Northern Species Reaching their Southerly Distribution Outposts in the Area, but Found only at Elevations in Excess of 1000 Ft.

Xyris montana = H
Juncus filiformis = G
Spiranthes Romanzoffiana = G
Mitella nuda = H
Fragaria canadensis = CH
Fragaria terra-novae = CH
Rubus pergratus = H
Pyrus sitchensis = MS
Viola nephrophylla = G
Viola Selkirkii = G
Viola renifolia = G
Moneses uniflora = G
Ledum groenlandicum = N
Vaccinium Brittonii = N
Adoxa Moschatellina = G
Valeriana uliginosa = G
Solidago macrophylla = H
Aster junceus = H
Petasites palmatus = H

Percentage of Growth-forms in Above List

(The percentages of the whole region included for comparison)

Growth-form	MG	MS	MC	N	CH	H	G	HH	T
Whole region.......	.52	4.03	7.18	3.51	5.29	33.29	20.23	11.74	13
Northern mountain species..........		5.26		10.52	10.52	31.57	42.1		

In the case of mesophanerophytes the percentage is obviously misleading, but the notable figure here, as in all those for the local flora area, is the high percentage of geophytes. It is often difficult to decide whether or no any given species belongs to the geophytes or hemicryptophytes, but errors of assignment to one or other of these groups should about equalize each other. It cannot be, then, that this abnormally high geophyte percentage is even partially explainable upon the assumption that many plants are incorrectly credited to the group, as there is no more reason why they should have been incorrectly assigned to this group than to any other.

The conclusion as to the climate of our area, as reflected in the spectrum of the whole flora is that the conditions seem more favorable here for the production of deep-rooted perennials of the bulb-bearing or rootstock type than any region as yet studied, and that the production of aquatics is relatively great. Figures for eastern Asia in this connection would be of interest. Such generalizations must mean very little as yet, because all such schemes of correlating climate and plant distribution deal with species rather than individuals. Biological spectra based on a census of individuals would very greatly alter the result. The chief value of this scheme of Raunkiaer's, and it is the most suggestive of all schemes yet devised for the purpose, is the opportunity it gives for comparison of one flora with another, for comparing certain elements of the same flora, and it has been used in studying even smaller categories of vegetation. To the ecologist and phytogeographer it opens up a wide field of investigation. Its value to the practical agriculturist and horticulturist must be apparent, as it can be applied as a criterion of hardiness and suitability of plants from one region for another. A study of the scheme from this standpoint would surely reveal much information of value to growers.

In the following four families, as illustrating the method, the species have been assigned to their respective growth-forms. In many cases it is not easy to assign the species, and much valuable work can be done along this line. It would be of the greatest service to know all the growth-forms as shown in certain areas, formations, associations and so forth.

IRIDACEAE

Iris versicolor = G
Iris primsatica = G
Sisyrinchium angustifolium = H
Sisyrinchium mucronatum = H
Sisyrinchium arenicola = H
Sisyrinchium gramineum = H
Sisyrinchium atlanticum = H

ALSINACEAE

Stellaria uliginosa = T
Stellaria pubera = H
Stellaria longifolia = T
Stellaria borealis = T
Cerastium nutans = T
Cerastium arvense = H
Cerastium arvense oblongifolium = CH
Sagina procumbens = T or biennial?
Arenaria caroliniana = CH
Arenaria stricta = CH
Arenaria groenlandica = CH
Arenaria lateriflora = H
Arenaria peploides } = stem succulents
Spergularia marina }

ROSACEAE

Physocarpus opulifolius = MC
Spiraea latifolia = N
Spiraea alba = N
Spiraea tomentosa = CH?
Gillenia trifoliata = G
Potentilla pumila = H
Potentilla canadensis = H
Potentilla canadensis simplex = G
Potentilla monspeliensis = T
Potentilla arguta = H
Potentilla palustris = CH
Potentilla tridentata = CH
Potentilla fruticosa = N
Fragaria vesca americana = CH
Fragaria virginiana = CH
Fragaria canadensis = CH
Fragaria terrae-novae = CH
Sanguisorba canadensis = H
Agrimonia gryposepala = H
Agrimonia rostellata = H
Agrimonia mollis = H
Agrimonia Bicknellii = H
Agrimonia striata = H
Agrimonia parviflora = H
Geum vernum = H
Geum virginianum = H
Geum canadense = H
Geum flavum = H
Geum strictum = H
Geum rivale = H
Waldsteinia fragarioides = H
Rubus 28 species all H
Rosa 7 species all N

HYPERICACEAE

Ascyrum stans = CH
Ascyrum hypericoides = CH
Hypericum Ascyron = H
Hypericum densiflorum = N
Hypericum adpressum = CH
Hypericum Bissellii = G
Hypericum ellipticum = G
Hypericum virgatum = G
Hypericum perforatum = G
Hypericum punctatum = G
Hypericum mutilum = T
Hypericum gymnanthum = T
Hypericum majus T? } perhaps
Hypericum canadense T? } biennial
Hypericum gentianoides = T
Hypericum virginicum = T

THE TEMPERATURE OF LEAVES OF PINUS IN WINTER[1]

John H. Ehlers

I. Introduction

There is evidence to show that the food reserve of decidous trees is at a maximum in autumn at the fall of the leaves, and that there is a gradual decrease in reserve material during the winter months to a minimum in the spring. For trees with persistent leaves, on the contrary, there is evidence to show that there is a gradual *increase* in reserve food during the winter, and that the maximum is not reached until just before the swelling of the buds in spring. Accepting the evidence as true leads to the conclusion that, while deciduous trees maintain their existence during the winter at the expense of the reserve food, trees with persistent leaves produce, by photosynthesis during the same season, food material not only for their maintenance, but in quantities sufficient for an accumulation of reserve. How they are able to do this in cold climates where the temperature rarely rises far above 0° C., and where the mean temperature for the winter months is several degrees below 0° C. is a problem that has not been solved.

It has been shown by investigators that broad leaves, *i. e.*, leaves of deciduous trees, exposed to tropical insolation and leaves exposed to summer insolation in temperate regions, may attain in still air a temperature 16° C. above the shade temperature of the air. Assuming that a similar condition holds for trees with persistent leaves, such as the conifers, during the cold days of winter, may not this offer a solution to the problem suggested above?

While a number of investigators have definitely determined the effect of solar radiation upon broad leaves for tropical regions, and for summer conditions in temperate regions, no such work has been done to find its effect upon persistent leaves under winter conditions. It was to fill this gap, *i. e.*, to determine the effect of solar radiation upon the temperature of persistent leaves under winter conditions, and its possible bearing upon photosynthesis and the accumulation of reserve food material during the same season, that this investigation was undertaken. The work was carried on in the Botanical Department of the University of Michigan during the winter months of 1912–1913 and 1913–1914. The problem was suggested to the author by Professor F. C. Newcombe, and was carried to completion under his supervision. To him my sincere thanks are due for helpful suggestions and kindly criticism. Grateful acknowledgment is also made to Professor H. M. Randall of the department of physics for valuable suggestions and help in setting up the apparatus, and for apparatus loaned by him; and to Professor N. H. Williams of the department of physics for the use of his laboratory and for helpful advice given.

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[1] Contribution No. 145 from the Botanical Department of the University of Michigan.

II. Historical

The papers of particular interest for the consideration of the problem under discussion may be grouped under three heads: (1) the internal temperature of foliage leaves; (2) photosynthesis at low temperatures; (3) accumulation of reserve food material by evergreen trees in winter.

1. *The Internal Temperature of Foliage Leaves*

Of interest here, merely because it is the first record of an investigation to determine leaf temperatures that could be found in botanical literature, is a paper by Rameaux (21) published in 1843. This investigator placed neighboring leaves, attached to the stems, one upon another until the layer was sufficiently thick to prevent any light from passing through, and then bent the layer around a mercury thermometer. No record of leaf temperatures is given, however, since the investigation was chiefly concerned with finding the temperature of stems.

Schumacher (27) was the next to attempt a determination of leaf temperatures. His method consisted in placing a thermometer against the lower side of a leaf exposed to solar illumination. At least two serious objections may be raised to this method: (1) Assuming a difference in temperature between the leaf and the surrounding air, only the small portion of the mercury bulb in contact with the leaf would be influenced by the leaf temperature; (2) the rays passing through the leaf would tend to warm the mercury regardless of the leaf temperature.

Askenasy (1) in 1874 made observations on the temperature attained by succulent plants when exposed to solar radiation. *Sempervivum* and *Opuntia* were used for this purpose. A mercury thermometer was laid against the upper surface of the leaf, or inserted in a cut made for that purpose. With the thermometer in the shade registering 31° C., the leaves of *Sempervivum* attained a temperature of 43.7° to 51.2° C., an excess of 20.2° C. over the air temperature. With leaves of *Opuntia* he obtained a temperature of 15° C. above the shade temperature. Thin leaves (*Aubrietia* and *Gentiana*) on the other hand reached a temperature of only 7° C. above that of the air. The difference in temperature attained by succulent and by thin leaves he ascribes to two causes: (1) the lower rate of transpiration of the succulent leaves; (2) their massive structure, exposing less surface in proportion to mass than thin leaves expose.

Worthy of mention because of the method employed is an investigation by Stahl (29) to determine the difference in temperature between the red and non-red parts of variegated leaves. Stahl seems to have been the first to apply a thermo-electric method to determine leaf temperatures. His apparatus consisted of a thermo-couple of German silver and copper, and a mirror galvanometer read by means of scale and telescope. The junctions were made spatulate in form and were pushed into the leaf lamina. Variegated succulent leaves were used. The source of light was a gas flame. Since no absolute temperatures are given his work needs no further mention here.

Under tropical insolation, Ewart (5) obtained with leaves of *Vanilla* and *Hoya* temperatures of 45° and 50° C. respectively. The leaves were suddenly bent around and pressed against a delicate mercury thermometer originally a little below the expected temperature. The results obtained compare fairly well with those obtained by Askenasy.

Ursprung (31) in the fall of 1901 made observations on the temperature of both succulent and thin leaves under natural illumination. The observations were made in the Basel Botanical Garden at temperatures ranging from 14° to 28° C. Ursprung rejected the thermo-electric method on the ground that the direct reading of temperatures with a mercury thermometer was more simple, and because of the difficulty of getting an instrument sufficiently sensitive over a wide range of temperatures. Thermometers with small cylindrical bulbs and graduated to .5 degrees over a range of 10° to 50° C. were used.

For finding the temperature of thin leaves, the thermometer was placed on the upper surface of the leaf. The sides of the leaf were folded over with the midrib as an axis, and fastened with a light wooden clamp. In handling, the leaves were kept from contact with the fingers by using a pad of wadding. In the case of succulent leaves a small hole, the exact diameter of the thermometer bulb, was bored and the bulb inserted. The results obtained for succulent leaves under strong solar illumination confirmed the findings of previous investigators, *i. e.*, for *Opuntia* 43.3° C., *Mamillaria* 43.5° C., and for *Sempervivum* 49.6° C. These were the maximal temperatures. The air temperatures were 27.5°, 28°, and 27.1° C. respectively. The temperatures found for thin leaves were much lower, *Ulmus montana*, *Betula alba*, and *Saxifrága crassifolia*, showing maxima of 0.7°, 2.3°, and 8.1° C. respectively, above the air temperature. Ursprung ascribes the lower temperature of thin leaves, as did Askenasy, to the very large surface exposed in proportion to the total mass of the leaf.

In 1905 four important papers appeared, important because more refined methods were used in the determination of leaf temperatures than had been employed by previous investigators. Of these, one, prepared by Miss Matthaei (14), was communicated to the Royal Society of London by F. Darwin in 1903. Miss Matthaei, in the course of her researches on vegetable assimilation and respiration, found it necessary to determine the internal temperature of the leaves used. For this purpose detached leaves of cherry laurel (*Prunus Laurocerasus*) held in a special leaf chamber and placed in a constant temperature bath, were exposed to light of varying intensity from an incandescent gas lamp. For strong illumination, a powerful Keith burner was used. The leaf was protected from direct heating effect by a system of circulating water. The temperatures were determined by means of a very fine thermo-couple of copper and constantan. One junction was inserted in the midrib of the leaf, the other, insulated by a rubber tube, was placed in a water bath the temperature of which could be varied at will. The electromotive force produced by the difference in temperature at the two junctions was read by means of a Thomson mirror-galvanometer, about ½ ohm in resistance. The deflections were read directly on the scale; no refinements, such as the use of a telescope, were considered necessary. This apparatus was found by calibration to be sensitive to 0.1° C. The temperatures were not read directly by means of the galvanometer, but by a zero method which was to adjust the temperature of the bath containing the control junction until the galvanometer showed no deflections. The temperature of the bath then indicated the leaf temperature. This method was easily accurate to within .5° C., which was considered sufficient for the purpose.

Under strong illumination from the powerful Keith burner, leaves of cherry laurel reached a temperature of 10° C. or more above the temperature of the bath.

The second of the above mentioned papers was presented before the Royal Society of London in March, 1905, by Brown and Escombe (4). The paper deals with investigations on the physiological processes of green leaves. It is interesting in this connection because of the method employed to determine leaf temperatures. The temperatures were arrived at by complicated calculations involving (1) the coefficient of absorption of radiation, (2) the specific heat of the leaf, (3) the energy expended in photosynthesis and respiration, (4) the thermal emissivity of the leaf, and (5) the effect of the wind velocity. Both attached and detached leaves of *Helianthus annuus* and *Senecio grandifolius* were used. Under full solar illumination, a maximum temperature difference between leaf and air of only 2° C. was found by this method. This work was done at air temperatures of 15° to 27° C.

In strong contrast to the results of Brown and Escombe are those obtained by Blackman and Matthaei (2) in their investigations of vegetable assimilation and respiration. Determinations of leaf temperatures under natural illumination, both with the leaf in open air and enclosed in a glass case, were made thermo-electrically. The apparatus and method used were identical with those used by Matthaei (14). For experiments in the open air detached leaves of cherry laurel were stretched on a frame, the leaf stalk dipping in a well of water. The thermo-junction was inserted in the midrib. Under brilliant insolation, temperatures varying from 7° to 16° C. above the shade temperature of the air were obtained. In diffuse light the leaves were found to be from 1° to 3° C. above the air temperature. Of the two methods—the complicated calculations of Brown and Escombe and the thermo-electric method of Blackman and Matthaei—the latter seems by far the more trustworthy. The sources of error are very much reduced.

In confirmation of the results obtained by Blackman and Matthaei are those obtained by Smith (28) in Ceylon. The apparatus and methods employed by Smith were practically the same as those used by Blackman and Matthaei and, therefore, need no further description. His results may be summarized in part as follows: (1) Thermo-junctions give the same result whether inserted in the lamina or in the midrib of the leaf; (2) "in still air, with the black bulb in vacuum thermometer at from 55° to 62° C., the air temperature in the shade being 25° to 28° C., leaves, whether thick and fleshy, or thin and pliable, when placed normal to the sun's rays, may reach a temperature of 15° C. above that of the surrounding air;" (3) "in the shade such leaves have an internal temperature varying from 1.5° C. below to 4° C. above that of the surrounding air;" (4) thickness and texture of leaf have little effect, but thick leaves require more time to reach the final temperature and cool off more slowly; (5) air currents and transpiration are important factors in reducing leaf temperatures.

2. *Photosynthesis at Low Temperatures*

While it has long been recognized that photosynthesis is influenced by the temperature to which the plant is exposed, comparatively little work has been done to determine the relationship between them. This is especially true of photosynthesis at low temperatures, such as prevail during the winter season in cold temperate regions. Until within recent years only a few more or less isolated determinations of the lowest temperature at which photosynthesis begins have been made.

Among the first investigators of photosynthesis at low temperatures was Boussingault (3) who observed an evolution of oxygen by *Pinus laricio* at a temperature of 0.5° to 2.5° C., and by meadow grasses at 1.5° to 3.5° C. It is interesting to note that Boussingault took into account the effect of solar radiation upon the leaf temperature. The plants with which he experimented were placed on the north side of a wall to protect them from the sun's rays during the experiment.

Heinrich (8) found that in *Hottonia palustris* carbon-dioxide assimilation began at 2.2° R., while Kreusler (11), experimenting with *Phaseolus vulgaris*, *Ricinus communis*, *Prunus Laurocerasus*, and *Rubus*, observed an assimilation of CO_2 at temperatures of − 0.9°, − 0.6°, − 2.2°, and − 2.4° C. respectively.

Jumelle (9), as a result of experiments with *Evernia Prunastri*, *Picea excelsa*, and *Juniperus communis*, makes the assertion that these plants, when exposed to sunlight at temperatures as low as − 30° to − 40° C., absorb CO_2 and evolve oxygen, while respiration ceases at − 10° C. He suggests that the absorption of heat along with the light rays by the exposed leaves may prevent the cell sap from being entirely frozen at even these low temperatures and that, in consequence, assimilation may proceed.

Pfeffer (20) offers in criticism of Jumelle's results the following: "Since, however, all respiration ceases at − 10° C. to − 12° C., it is manifestly impossible that any assimilation of carbon dioxide can take place at − 40° C., for CO_2 assimilation is a vital process involving protoplasmic activity." Jumelle's methods are generally considered faulty by later investigators, and not much credence is placed in his results. For criticism of his methods the reader is referred to Matthaei (15) and to Ewart (7).

Miyaké (18) has found that the leaves of a large number of evergreen plants, including trees, shrubs, and herbacous plants growing in the vicinity of Tokyo, contain more or less starch during the winter. To determine whether this starch was the product of photosynthesis in winter, or whether it was stored there as suggested by Sachs (25), he excluded light from several plants (*Thea japonica*, *Fetsia japonica*, *Cinnamomum Camphora*, *Pinus Thunbergii*, and *Abies firma*), some in the open, others in a dark chamber the temperature of which varied between 1° and 7° C., until the starch had disappeared, and then exposed them to sunlight. Microscopical tests showed the reappearance of starch within five hours after exposure. The temperatures were: for *Thea*, minimum 2.6° C., maximum 8.6° C., mean 3.1° C.; for *Fetsia*, minimum 0.8° C., maximum 9.7° C., mean 3.4° C. He concludes that starch is formed by photosynthesis in winter and that its translocation occurs in the same season. This is further evidence, not only of photosynthesis in winter, but also of the accumulation of reserve material. The temperatures given were taken from the records of the Meteorological Observatory of Tokyo, and, presumably, are shade temperatures and, therefore, do not represent the true temperature of the leaf. As will be shown later, the absorption of the sun's radiations by the leaf will increase the internal temperature of the leaf from 3 to 10 degrees Centigrade on bright winter days.

The next contribution of importance was by Ewart (6). In a paper on assimilatory inhibition he devotes a part to experiments on assimilation at low temperatures. He used the bacterium method.

With this method the lowest temperatures at which determinations can be made is approximately 1° C. Parts of plants that were growing in the open and had been exposed for several weeks to temperatures often reaching – 15° C. were thawed and examined at 1° C. The following plants among others, although most of their leaves remained alive, showed no power of assimilation at 1° C.: *Ilex Aquifolium*, *Buxus sempervivens* var. *arborescens*, *Pinus montana*, *Taxus baccata*, *Thuya occidentalis*, and *Juniperus Sabina*. Experiments were also made with tropical, subtropical and water plants. His results are summarized as follows: "It appears that all evolution of oxygen ceases in tropical plants between 4° C. and 8° C.; in warm temperate, subtropical and water plants between 0° C. and 2° C., whilst in cool temperate, arctic, and alpine plants assimilation only ceases when the plants are frozen, *i. e.*, at a few degrees below 0° C." The last case is, presumably, an inference, since he offers no experimental proof to substantiate it. He ascribes the cessation of assimilation in cool temperate, arctic, and alpine plants to physical causes, *i. e.*, the withdrawal of water from the protoplasm to form ice crystals and the consequent desiccated condition of the tissue.

The most important contribution to the question under consideration was made by Matthaei (14). Determinations of the rate of assimilation through a range of temperatures from – 6° C. to 45° C. were made. The leaves of cherry laurel, *Prunus Laurocerasus* var. *rotundifolia*, were used for this purpose. Her work differs in two respects from that of all previous investigators: (1) In that careful attention was given to keeping the material under uniform conditions before the experiment; (2) in that precautions were adopted for obtaining the true internal temperature of the leaf. The leaf temperatures obtained and the method of determining them have already been briefly stated. Of the results obtained those which are of particular interest here are:

1. The minimum at which assimilation could be observed. This was – 6° C. Disregarding Jumelle's finding as untrustworthy, this is the lowest temperature at which photosynthesis has been observed. The author further suggests that, for such cold-enduring plants as the conifers, assimilation may take place at temperatures considerably lower.

2. The assimilation curve. This curve shows the maximal amount of assimilation at all temperatures from – 6° C. to 43° C. From

– 6° C. to 38° C. the curve is convex to the temperature abscissa, the maximal amounts increasing rapidly as the temperature increases. For example, the maximal amounts increase from 0.2 mmg. of CO_2 per hour for 50 sq. cm. of leaf surface at – 6° C. to 3.8 mmg. at 9° C. The conclusion is that, other conditions being favorable, assimilation increases directly with the temperature; that for each temperature there is a definite amount of assimilation beyond which a further increase in light intensity produces no effect except in so far as it increases the internal temperature of the leaf. A greater assimilation can be obtained only by increasing the temperature. The author considers temperature, therefore, as the fundamental condition governing assimilation, intensity of light as well as percentage of CO_2, since they are always present in sufficient quantities, being of secondary importance.

3. *Accumulation of Reserve Food Material by evergreen Trees in Winter*

In 1904 Sablon (23) published the results of his investigations on the reserve material of deciduous trees. He found that the reserve carbohydrates of these trees reached a maximum in autumn at the fall of the leaves at the end of the period of active assimilation. During the winter the reserve decreased a little, while in the spring during the formation of new shoots there was a decided diminution—a fall to the minimum. Two years later (24) he published the results of an investigation of the reserve materials of evergreen trees (les arbres a feuilles persistantes). The species used were *Quercus Ilex*, *Pinus laricio*, *Larix europoea*, and *Evonymus japonicus*. The determinations in both investigations were made by chemical analysis. The results obtained in this investigation were strikingly different from those obtained with deciduous trees. Instead of the maximum appearing in autumn, there is a constant increase in reserve material during the winter, and a maximum is reached only at the beginning of spring. To quote directly: "Pendent l'hiver, en effet, la végétation est suspendu, par conséquent la dépense de réserve est faible; d'autre part l'assimilation du carbone se poursuit et l'on sait que l'abaissement de la température affaiblet beaucoup mains l'assimilation que la respiration. Il est donc naturel que l'hiver soit pour les arbres à feuilles persistantes une périod de formation de réserve." In other words, in trees with persistent leaves photosynthesis is not only sufficient for the maintenance of the tree, but there is actually an increase in the reserve material during the "rest period" so called. Mer (17) thirty years earlier working with the leaves of *Hedera* came to a similar conclusion. The experiments of both of these investigators were made with material growing in the open—Mer at Paris, Sablon at Toulouse. Unfortunately neither gives any data as to temperatures. How far their results may be applied in a given locality depends upon the factors governing photosynthesis in that locality. That they hold for the greater part of Japan has been shown conclusively by Miyaké (18) in the investigations cited above.

III. METHODS

A. Preliminary Work in 1912–13

1. *Material and Location.*—Since the purpose of this investiagtion was to find the internal temperature of pine leaves in winter under as nearly natural conditions as possible, cut branches were avoided and leaves *in situ* used. As best suited for the purpose, the species *Pinus laricio austriaca* Endl. was selected. The leaves of this species are, in cross section, the largest of the conifer leaves available in this locality.

The tree used during the preliminary work is about 2.5 meters high and is located in the university arboretum about a mile from the university campus. A small shed was built near the tree to house the apparatus.

2. *Method.*—The character of the leaves and the conditions under which the work was to be carried out made a thermo-electric method the only method possible. In the preliminary work an attempt was made to interpret directly into temperature differences, by means of a d'Arsonval galvanometer, telescope, and scale, the electromotive force produced by the difference in temperature of two thermo-junctions, one embedded in the leaf tissue, the other in a thermos-bottle kept at a known temperature—usually at 0° C. This method was, however, found unsatisfactory. The changes in the resistance of the lead wires and the wires connecting the leads with the galvanometer in the shed, as well as the changes in resistance in the galvanometer itself, due to changes in atmospheric conditions, made new calibrations necessary not only each day, but sometimes several times a day. After a thorough test this method was abandoned.

B. Final Experiments

1. *Material and Location.*—When the work was resumed in the autumn of 1913 the station in the arboretum was abandoned. An Austrian pine growing on the university campus was selected for the final experiments. This tree is about 40 years old and about 15 meters high. It is protected from direct winds on the north and west by the library, and on the south by the physics laboratory. To give the operator access to the leaves and to hold the outdoor part of the apparatus, a wooden platform 4 meters high was constructed. Upon this platform a wooden screen was set up to protect the apparatus from the sun's rays. Three pairs of heavy insulated copper wires connected the platform with the ground floor of the physics laboratory where the measuring apparatus was kept.

2. *Method.*—The first two months were spent in trying out various methods. A potentiometer method was finally adopted as best suited for the purpose. The principle of the method consists in opposing over the slide wire of a Wheatstone bridge the fall of potential due to a suitable current from a cell against the electromotive force produced by the difference in temperature between the two junctions, one of which is in the leaf. By moving the sliding contact, the portion of the bridge wire included in the galvanometer circuit (*A* to *B*, figure 1) may be changed until the fall of potential over this portion is equal and opposite to the resultant E.M.F. due to difference in temperature of the junctions. This condition is indicated by a zero deflection of the galvanometer. The displacement along the wire per degree temperature difference is determined empirically by placing the junctions in baths of known temperature.

The great advantage of this method over the first one employed is that, since it is a zero method, no current flows in the galvanometer circuit and variations in resistance due to temperature changes and other causes may be neglected. Furthermore, its accuracy is independent of any assumption of proportionality between galvanometer deflection and current.

3. *Apparatus.*—The apparatus in its essential parts consisted of a slide wire Wheatstone bridge, galvanometer, storage cell, resistance boxes, rheostat, and voltmeter. Figure 1 shows the arrangement. The galvanometer used was of the d'Arsonval type with a resistance of 30.6 ohms, and was made by the Eberbach and Son Co., of Ann Arbor, Michigan. The Wheatstone bridge was made by the same

firm. The cell used to furnish the current for the potentiometer circuit (II, figure 1) was an ordinary storage cell with an E.M.F. of 2 volts. This cell proved entirely satisfactory, giving a uniform current throughout the experiments. As a check upon the cell, a Weston voltmeter was connected across the terminals of the potentiometer circuit. By throwing a knife switch, the voltage applied to this circuit of constant resistance could be measured, and any variations or weakening of the cell detected. Variations could be corrected by varying the resistance of a small rheostat which was placed between the cell and the potentiometer. It was not found necessary to use it however.

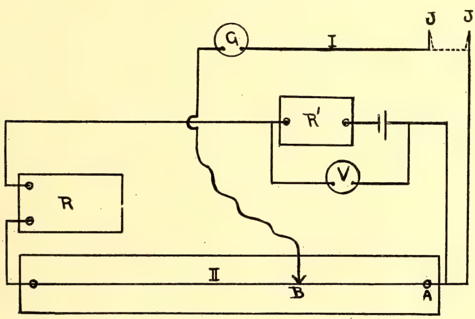


Figure 1.

I Galvanometer Circuit.
II Potentiometer Circuit.
B Sliding Contact.
G Galvanometer
J, J' Thermo-junctions
V Voltmeter
R, R' Resistance Boxes.

4. *Thermo-Junctions.*—The thermo-junctions were made of *advance* (a trade name for constantan, which is an alloy of copper and nickel) and copper wire obtained from the Driver, Harris Co., of Harrison, N. J. The advance wire was 0.07 mm. in diameter, the copper slightly

larger – 0.09 mm. The wires were carefully soldered, and all junctions having rough or thick joints rejected. A thin coating of shellac was given the junctions to protect them against oxidation and any possible action of the leaf juices.

5. *Method of Insertion into the Leaf.*—The leaves of *Pinus laricio austriaca* grow in bundles of two. The leaf in cross section represents roughly a semi-circle in outline about 1.5 mm. in its shortest diameter. To insert the junctions into the leaf at temperatures ranging from 0° to – 17° C. was attended with considerable difficulty and many junctions were broken, especially in the beginning of the experiments. The procedure was to thread the lead wire through a very fine steel needle and draw the wire through the leaf until one of the junctions was embedded in the tissue. To prevent too great a loss of heat by conduction along the lead wires, as much of the wire as possible should be embedded in the leaf tissue. Drawing the wire through at right angles to the long axis of the leaf would not, therefore, give the best results. Drawing the wire through the leaf lengthwise was found impracticable because of the firmness of the tissue. Attempts to do this usually resulted either in the splitting of the epidermis, or the breaking of the leaf. Placing the junction and lead wires between two leaves fastened together flat surface to flat surface did not give good results. The method finally adopted was to fasten the two leaves together by means of two single turns of very fine wire placed about 1 cm. apart, the wire covered with white insulation. The leads were drawn through both leaves at an angle of 45° with their long axis. Care was observed to leave the junction embedded in the leaf toward the sun and as near its center as possible. This gave a contact surface of about 1 mm. for the advance lead wire and from 3 to 4 mm. for the copper—the better conductor of the two. The second junction was left exposed to the air and shaded from the sun's rays by a wooden screen. The junctions, therefore, registered directly the differential temperature between leaf and surrounding air.

6. *Position of the Leaf.*—The leaves of the pines assume no definite position with relation to the sun's rays. In all experiments, unless otherwise stated, a leaf normal to the sun's rays, or as nearly normal as could be found, was selected. Two sets of junctions, each in a different leaf, were constantly in use—the one a check upon the other. A double-pole double-throw switch on the table beside the operator made it possible to read the temperatures of the two leaves in rapid

Table I.

Calibration of Apparatus

Temperature			Bridge Reading in Cm.	Displacement per Degree C. Temp. Diff.	Average Displacement per Degree Temp. Diff. in Cm.	Degrees C per Cm. Displacement
Junction *A*	Junction *B*	Temp. Diff.				
−9.82°	−15.40°	5.58°	44.6	7.99	8.00	0.1250°
−9.82	−15.10	5.28	42.1	7.97		
−9.81	−15.00	5.19	41.5	7.99		
−7.30	−14.85	7.55	60.7	8.04		
−7.18	−14.70	7.52	60.4	8.03		
−4.87	−10.40	5.53	44.7	8.08	8.10	0.1234
−4.94	−10.00	5.06	41.0	8.10		
−5.00	− 9.92	4.92	39.8	8.09		
−5.11	− 9.68	4.57	37.1	8.12		
−5.13	− 9.65	4.52	36.7	8.12		
0	− 6.52	6.52	53.6	8.20	8.22	0.1216
0	− 6.58	6.58	54.0	8.21		
0	− 6.60	6.60	54.3	8.23		
0	− 6.60	6.60	54.2	8.21		
0	− 5.65	5.65	46.5	8.23		
0	− 5.60	5.60	46.0	8.21		
0	− 5.50	5.50	45.4	8.25		
0	− 5.45	5.45	45.0	8.25		
5.00	0	5.00	41.6	8.32	8.30	0.1205
5.00	0	5.00	41.6	8.32		
5.00	0	5.00	41.5	8.30		
5.07	0	5.07	42.0	8.28		
5.28	0	5.28	43.8	8.28		
5.48	0	5.48	45.4	8.29		
5.62	0	5.62	46.5	8.27		
7.70	0	7.70	64.2	8.34		
3.00	0	3.00	25.0	8.33		
13.25	10.00	3.25	27.6	8.49	8.50	0.1176
13.52	10.00	3.52	29.9	8.52		
14.60	10.00	4.60	39.2	8.52		
14.61	10.00	4.61	39.3	8.52		
14.67	10.04	4.63	39.4	8.51		
14.80	10.14	4.66	39.6	8.50		
13.33	9.75	3.58	30.3	8.46		
26.20	18.61	7.59	65.8	8.64	8.65	0.1156
26.20	18.62	7.58	65.6	8.64		
26.20	18.62	7.58	65.6	8.64		
26.20	18.64	7.56	65.5	8.66		
26.20	18.64	7.56	65.5	8.66		
26.62	18.68	7.94	68.8	8.67		
27.27	18.68	8.59	74.3	8.64		
27.26	18.71	8.55	74.0	8.65		
27.95	18.72	9.23	80.0	8.67		
27.90	18.72	9.18	79.5	8.66		

TABLE I.—*Continued*

Temperature			Bridge Reading in Cm.	Displacement per Degrees C. Temp. Diff.	Average Displacement per Degree Temp. Diff. in Cm.	Degrees C. per Cm. Displacement
A	*B*	Temp. Diff.				
23.57	20.00	3.57	31.1	8.71	8.71	0.1148
23.54	20.00	3.54	30.9	8.73		
23.49	20.05	3.44	30.0	8.72		
23.40	20.05	3.35	29.2	8.72		
23.36	20.05	3.31	28.9	8.73		
25.15	20.15	5.00	43.4	8.68		
26.10	20.15	5.95	51.8	8.70		
26.13	20.18	5.95	51.9	8.72		
26.22	20.20	6.02	52.3	8.69		

succession. By comparing fresh leaves with others in which the junction had remained embedded for several days, it was found that the same leaf could be used 3 or 4 days in succession without any appreciable effect upon the results.

7. *Calibration of Apparatus.*—In calibrating the apparatus the junctions were tied to the bulbs of the thermometers, the leads separated from one another by small glass tubes. Junctions and thermometers were then thrust into glass tubes containing kerosene oil and the tubes immersed in constant temperature baths. The leads of the junctions were connected with the apparatus by means of copper wires and copper binding posts. By means of resistance boxes the current from the cell was reduced until a displacement on the bridge of 100 cm. was equivalent to a temperature difference at the junctions of approximately 12° C. Readings were taken with the colder junction at temperatures varying from 18° to 20° C., while the warmer junction ranged from 0.5° to 10° C. higher. More than 50 readings gave an average displacement on the bridge of 8.66 cm. per degree centigrade temperature difference, or 0.1155° C. per centimeter displacement. But experiments showed that this factor could not be used for temperature differences at 0° C. and below, the error varying from 0.05° to 0.5° C. Calibrations were therefore made at various points through a temperature range of 35 degrees, with the colder junction at − 15°, − 10°, − 5°, 0°, 10°, 18° and 20° C., the warmer junction varying from three to eight degrees higher in each case. Standard thermometers, one graduated to 0.2° C. the other to 0.1° C. were used for temperatures from 0° to 20° C. The low temperatures were obtained by means of two Beckmann thermometers. With these the temperatures could be read without lifting the bulb in the freezing mixture. The results are given in Table I.

If these values are plotted against the temperatures as ordinates, a curve is obtained which, for all practical purposes is a straight line, the deviation at − 5° C. being negligible. From this curve (figure 2), by interpolation, values can be obtained for any temperature between − 20° and 20° C. The two columns of figures at the right of figure 2 contain these empirical numbers with the computed numbers interpolated.

8. *Sensitiveness of Apparatus.*—The galvanometer was found readily sensitive to a displacement on the bridge wire of two millimeters. Taking the factor obtained for 0° C. as an example, the apparatus is sensitive to $.2 \div 8.30 = 0.024°$ C., which is amply sufficient for the purpose of this investigation. The apparatus may be made still more sensitive by decreasing further the current from the cell and increasing the sensitiveness of the galvanometer correspondingly.

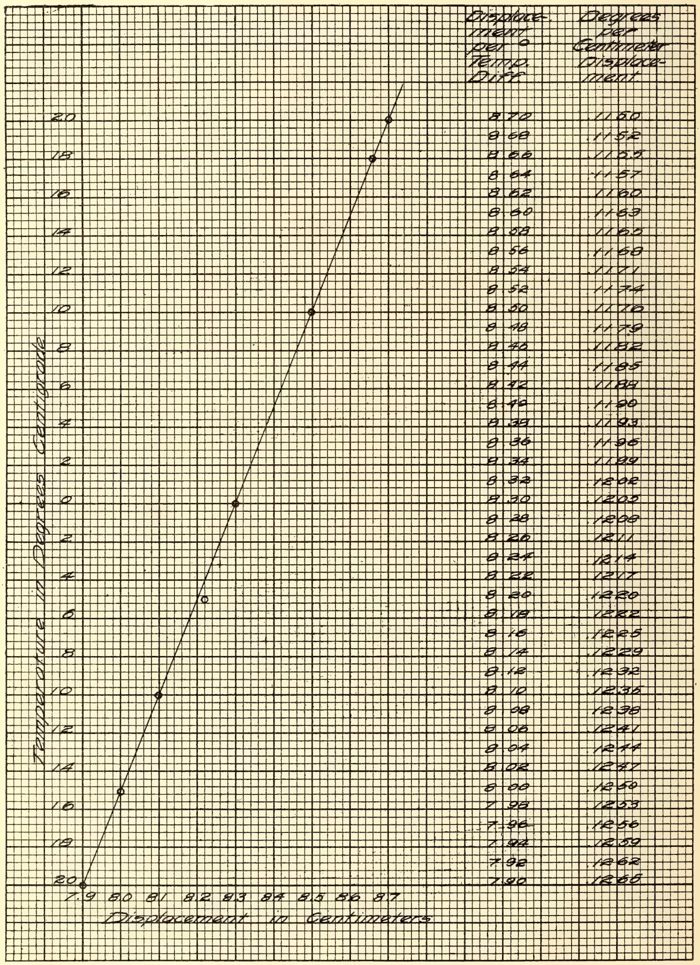
9. *Accuracy of the Method.*—To determine whether the theoretical sensitiveness of the apparatus would hold in practice, tests were made in December and January, and again in March at the close of the experiments. The method consisted in placing the junctions in baths of known temperatures, finding the corresponding displacement on the bridge wire, computing the temperature by applying the proper factor obtained from the curve, and then comparing the calculated with the actual temperature difference. The results are given in Table II.

Table II shows representative readings taken from a large number. The average error of the December and January readings is only 0.0155°; the greatest for any one reading 0.055°. For March the average error is 0.0233°; the greatest 0.056°.

10. *Uniformity of the Junctions.*—Table III gives the results of an experiment to test the uniformity of the junctions used. Two sets of junctions were placed in the same baths. By throwing a switch, changes from one set to the other could be made in rapid succession. A number of other tests gave results similar to those in the table.

The greatest difference in displacement is 2 mm. This, at the temperature difference given (5.04°) represents an error of 0.023° C., or .4 per cent.

11. *Sources of Error.*—The possible sources of error in this method are: (1) Variation in the current due to weakening of the cell; (2) the



Displacement per ° Temp. Diff.	Degrees per Centimeter Displacement.
8.70	.1150
8.68	.1152
8.66	.1155
8.64	.1157
8.62	.1160
8.60	.1163
8.58	.1165
8.56	.1168
8.54	.1171
8.52	.1174
8.50	.1176
8.48	.1179
8.46	.1182
8.44	.1185
8.42	.1188
8.40	.1190
8.38	.1193
8.36	.1196
8.34	.1199
8.32	.1202
8.30	.1205
8.28	.1208
8.26	.1211
8.24	.1214
8.22	.1217
8.20	.1220
8.18	.1222
8.16	.1225
8.14	.1229
8.12	.1232
8.10	.1235
8.08	.1238
8.06	.1241
8.04	.1244
8.02	.1247
8.00	.1250
7.98	.1253
7.96	.1256
7.94	.1259
7.92	.1262
7.90	.1265

FIG. 2 Curve showing displacement in centimeters on wheatstone bridge. The temperatures from zero down the column are all minus.

TABLE II

Date	Temperature			Displacement in Cm.	Calculated Temp. Diff. in Deg. Cent.	Error
	A	*B*	Actual Difference			
Dec. 27	24.53°	18.38°	6.15°	53.2	6.149°	0.001°
	24.52	18.39	6.13	52.9	6.115	0.015
	26.25	18.40	7.85	68.3	7.895	0.045
	24.65	18.85	5.80	50.2	5.803	0.003
	24.62	18.85	5.77	49.8	5.757	0.013
	23.35	18.90	4.45	38.6	4.462	0.012
	23.32	18.93	4.39	38.0	4.392	0.002
Dec. 28	20.21	18.04	2.17	18.8	2.173	0.003
	21.86	18.02	3.84	33.2	3.838	0.002
	19.19	18.16	1.03	8.9	1.029	0.001
	19.13	18.15	.98	8.5	.983	0.003
Jan. 5	8.00	0.00	8.00	66.7	8.037	0.037
	5.00	0.00	5.00	41.6	5.013	0.013
	2.97	0.00	2.97	24.7	2.976	0.006
Jan. 11	−7.40	−14.68	7.28	58.4	7.293	0.013
	−7.18	−14.70	7.52	60.4	7.543	0.023
	−7.05	−14.00	6.95	55.6	6.950	0.000
	−9.25	−16.67	7.42	58.7	7.365	0.055
	−9.80	−17.03	7.23	57.3	7.198	0.032
	−5.05	−10.00	4.95	39.9	4.924	0.026
					Average error	0.0155
Mar. 22	4.15	0	4.15	34.2	4.118	0.032
	4.18	0	4.18	34.8	4.190	0.010
	4.28	0	4.28	35.7	4.298	0.018
	4.38	0	4.38	36.4	4.383	0.003
	4.45	0	4.45	36.6	4.406	0.056
	4.56	0	4.56	37.9	4.563	0.003
	4.77	0	4.77	39.7	4.789	0.019
	5.18	0	5.18	42.8	5.153	0.027
	5.34	0	5.34	44.2	5.322	0.018
	5.45	0	5.45	45.0	5.418	0.032
	5.50	0	5.50	46.0	5.538	0.038
					Average error	0.0233

formation of secondary couples; (3) heat due to wounding of the leaf tissue; (4) loss of heat by conduction along the lead wires.

(1) *Variation of the Current from the Cell.*—To guard against any variation of the current from the cell, a Weston voltmeter and a rheostat were connected across the terminals of the potentiometer circuit as already described. The simple throwing of a switch enabled the operator to detect any change in the voltage applied to the potentiometer circuit. Corrections could then be made by varying the resistance of the rheostat.

TABLE III

COMPARISON OF JUNCTIONS

Date	Temperature			Displacement in Cm.		Diff. in Mm.
	A	*B*	Diff.	Junct. No. 1	Junct. No. 2	
Jan. 3	22.85°	20.42°	2.43°	21.0	20.9	1
	22.90	20.42	2.48	21.5	21.4	1
	23.04	20.48	2.56	22.2	22.1	1
	24.10	20.53	3.57	31.0	31.0	0
	24.10	20.53	3.57	31.0	31.0	0
	24.09	20.55	3.54	30.8	30.7	1
	25.60	20.56	5.04	43.7	43.5	2
	25.57	20.57	5.00	43.4	43.4	0
	26.58	20.56	6.02	52.3	52.3	0
	26.55	20.60	5.95	51.9	51.8	1
Jan. 4	20.28	19.24	1.04	9.0	9.0	0
	19.60	19.21	.39	3.4	3.3	1
	19.45	19.15	.30	2.7	2.6	1
	19.50	19.17	.33	2.9	2.9	0
	29.80	19.04	10.76	93.2	93.2	0
	29.78	19.04	10.74	93.1	93.0	1

(2) *The Formation of Secondary Couples.*—Any difference in the composition of the metals at the connections, or any slight difference in the composition of the metal in the wires themselves may, if there is a difference in temperature at those points, cause the formation of secondary couples. For the purpose of this investigation the latter case may be neglected, as the error arising from this source would be exceedingly small. To guard against secondary couples at the connection of the lead wires with the heavy copper wires, copper binding posts were used and the joints placed as closely together as practicable to insure the same temperature for both. The connections with the apparatus in the laboratory were all under uniform temperature conditions throughout the experiments and, consequently, the danger from secondary couples at those points was reduced to a minimum.

(3) *Heat Due to the Wounding of the Leaf Tissue.*—The development of heat in wounded tissue is due to an increased respiration of the injured part. Tiessen (30) found that the rise in temperature due to wounding increases with the extent of the wound; that its duration varies from one half to three days; and that its absolute value varies from 0.02° to 0.08° C. with an average of 0.04° C. Richards (22) has shown that a curve plotted for the heat developed in wounded tissue corresponds in the main to that of the respiration intensity under the

same conditions. Now since respiration is but feeble at 0° C. and decreases rapidly as the temperature falls [Maximow (16), Matthaei (14)], and since nearly all the readings were taken at 0° C. and below, and since the wound made in the leaf was very small, any increase in temperature due to wounding would not be appreciable.

(4) *Loss of Heat by Conduction Along the Lead Wires.*—This was, in all probability, the source of greatest error and could not be entirely overcome. As already stated, two leaves were fastened together and the lead wires drawn through them at an angle in order to give as large a contact surface as possible. To determine what the loss from this source might be, the following experiment was made. A small glass tube 15 cm. long with a bore of 5 mm. was furnished with two side arms and two minute openings. Three thermo-junctions, such as were used in taking the leaf temperatures, were inserted in this tube as shown in figure 3. Through this tube kerosene oil was siphoned from a bath kept at a temperature of 10° C. above the room temperature. The leads of junction 1 were in contact with the heated oil the entire length of the tube, and this junction should therefore record accurately the temperature of the flowing oil. The leads of junction 2, on the other hand, were in contact with the oil for a distance of .5 mm. to 1 mm. for the advance lead and 4 mm. for the copper—a condition similar to that of the junction in the leaf. By connecting junctions 1 and 2 with the potentiometer, any difference in temperature between these junctions due to loss of heat by conduction from junction 2 could be measured. By connecting junctions 1 and 3 in the same way the temperature gradient between the flowing oil and the surrounding air could be determined. A three-way switch enabled the operator to measure both in rapid succession. Repeated trials gave an average displacement on the bridge for junctions 1 and 3 of 84.5 cm. which at the room temperature was equivalent to a temperature difference of 9.7° C. Junctions 1 and 2 gave an average displacement of .36 cm. This repre-

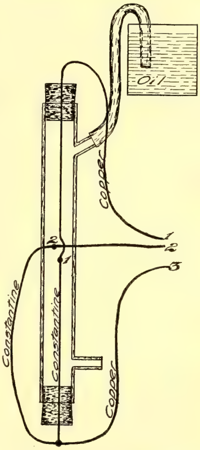


FIG. 3. Showing the details of the three thermo-junctions.

sents a temperature difference between these junctions of 0.04° C., *i. e.*, enough heat is lost by conduction from junction 2 to reduce its temperature 0.04° C. below the true temperature of the oil. When it is borne in mind that the temperature gradient between air and oil in this experiment was 9.7° C., and that the maximum temperature gradient between leaf and air under natural conditions was 8.83° C. and the average considerably below this, it is evident that the error arising from loss of heat by conduction along the lead wires is also negligible. Furthermore it is compensated for in part by the very small increase in temperature due to wounding.

(5) *Absorption of Radiation by the Junction in the Leaf.*—It may have occurred to the reader that the rise in temperature indicated by a junction placed in a leaf exposed to direct sunlight may be due in part to the absorption of radiation by the junction itself. Smith (28) has shown that this is not true. By alternately shading a junction by means of a small piece of pith and exposing it to direct sunlight, he found that direct sunlight had no effect upon the junction whatever. He concludes from his experiment that "the rise of temperature of a junction registered when it is placed in a leaf exposed to direct sunlight must all be due to the absorption of radiation by the leaf, and no part of it to the absorption of radiation by the junction itself." Smith's result was accepted by the author and no experiments were made to verify it.

12. *Meteorological Data.* (1) *Air Temperature.*—In the earlier experiments the air temperature was obtained with a mercury thermometer graduated to degrees Centigrade. Later a registered thermometer graduated to .1° C. was used. No attempt was made to read the air temperature beyond one tenth degree. The thermometers were hung behind the screen upon the platform.

(2) *Solar Radiation.*—For purposes of comparison, the intensity of the solar radiation was also taken. For this a black bulb in vacuum thermometer graduated to the Fahrenheit scale was employed. It was placed on the platform near the leaf. For the sake of uniformity the readings were converted into degrees Centigrade.

(3) *Velocity of the Wind.*—For this a self-registering anemometer making contact every one tenth mile was used. The miles of wind per five minute periods could be determined. The anemometer was placed upon the platform near the leaf; the registering device in the laboratory.

(4) *Relative Humidity.*—The relative humidity was obtained from the records of the university observatory situated about one half mile from the campus. The values given represent the average from 7 a.m. to 7 p.m.

IV. Experimental Data

A. Leaf Temperature under Winter Conditions

The collection of data began early in January. Of the mass of data obtained only a part can be given. The results shown by the tables following in the text may be taken as representative.

The readings, as a rule, were taken from one half to three minutes apart for a period of five to ten minutes. Then the air temperature, radiation, and the wind velocity were recorded for that period. To save space, the average of the readings for the period is given, as a rule. In a few cases all the readings are given to show the rapidity of temperature changes within the leaf.

The readings of the black bulb in vacuum thermometer are the maxima for the period unless otherwise indicated.

The wind velocity given in column 9 is the average for the period as recorded by the anemometer on the platform and has only general value. Since the tree, partly sheltered from direct winds by the neighboring buildings, was subject to sudden gusts and eddy currents, some of which were vertical and did not, therefore, affect the anemometer, the velocity given does not necessarily represent the true velocity at the moment the leaf temperatures were taken.

Column 4 in the tables gives the differential temperature between the leaf and the shade temperature of the air. The values are obtained by multiplying the bridge readings by the factor (see figure 2) indicated by the shade temperature given in column 5. Column 8 gives the actual temperature of the leaf. The values are obtained by adding the differential temperature to the air temperature. Column 7 gives the difference between the shade temperature and that recorded by the black bulb in vacuum thermometer—a better standard for comparison than the values in column 6. In general, the differential temperature between leaf and air increases as the radiation increases. When this is not the case, an explanation can usually be found by referring to the wind velocity recorded in column 9.

The data presented here are given to show: (1) the effect of full sunlight; (2) the effect of one fourth to one half full sunlight; (3) the effect of diffuse light; (4) the leaf temperature at night; (5) the effect of strong air currents; (6) the temperature of leaves at different angles to the sun's rays.

(1) *The Effect of Full Sunlight.*—Unfortunately no data on the effect of full sunlight in still air could be obtained. Low temperature, still air, and full sunlight are a combination of conditions that rarely obtains. The nearest approach to these conditions was found on February 24. The data obtained are given in Table IV.

TABLE IV

February 24, 1914

Clear. Light breeze. Relative humidity 87%

1	2	3	4	5	6	7	8	9
Time of Observation	No. of Observations	Aver. Bridge Reading in Cm.	Calculated Temp. Diff.	Shade Temp.	Black Bulb in Vacuum	Diff. Columns 5 and 6	Actual Leaf Temp.	Wind Velocity Miles per Hr.
9.00– 9.02	4	26.1	3.26°	−15.0°	5.6°	20.6°	−11.74°	1.2
9.21– 9.24	4	48.4	6.04	−14.1	14.4	28.5	− 7.06	1.2
9.28– 9.33	7	44.4	5.54	−14.0	20.0	34.0	− 8.46	2.4
10.02–10.04	4	60.4	7.51	−13.0	20.6	33.6	− 5.49	.4
10.50–10.55	7	44.0	5.46	−12.2	25.6	37.8	− 6.74	.8
11.00	1	56.0	6.96	−12.5	28.3	40.8	− 5.54	1.2
11.005	1	37.2	4.52				− 7.98	
11.01	1	50.8	6.31				− 6.19	
11.015	1	38.2	4.75				− 7.75	
11.02	1	64.0	7.95				− 4.55	
11.025	1	59.6	7.41				− 5.09	
11.03	1	46.5	5.78				− 6.72	
11.04	1	40.0	4.97				− 7.53	
11.05	1	54.0	6.71				− 5.79	
11.08–11.12	4	58.1	7.21	−12.0	28.9	40.9	− 4.79	1.2
11.25–11.32	8	56.5	7.00	−11.1	28.9	40.0	− 4.10	.9
11.29–12.35	7	57.6	7.11	−10.2	29.4	39.6	− 3.09	.5
2.35– 2.40	8	41.5	5.13	−10.0	11.7	21.7	− 4.87	.3
		Averages	6.04	−12.4		33.8	− 6.36	1.0

With the shade temperature of the air varying from − 15° to − 10° C. the black-bulb thermometer registering from 20.6° to 40.9° C. higher, and the wind velocity varying from 0.3 to 2.4 miles per hour, the average differential temperature between leaf and air for the entire time during which the readings were taken was 6.04° C., *i. e.*, the leaf maintained, under the conditions given, a temperature of 6.04° C. higher than the air. This is shown by the averages of columns 4, 5, and 8. The maximum temperature difference for the day was 7.95° C.

The following Table V shows a maximum leaf temperature of 8.83° above that of surrounding air, the air temperature in this series of observations being considerably higher than that shown in Table IV.

TABLE V

February 27, 1914

Clear. Light breeze. Relative humidity 66.5%

1	2	3	4	5	6	7	8	9
Time of Observation	No. of Observations	Aver. Bridge Reading in Cm.	Calculated Temp. Diff.	Shade Temp.	Black Bulb in Vacuum	Diff. Columns 5 and 6	Actual Leaf Temp.	Wind Velocity Miles per Hr.
8.00[2]	5	5.7	.69°	− 2.0°	7.2°	9.2°	−1.31°	1.2
9.15	4	18.6	2.24	0	18.9	18.9	2.24	1.2
9.25	5	16.4	1.97	0.8	20.6	19.8	2.77	3.6
9.33	8	32.5	3.90	1.5	23.9	22.4	5.40	1.0
10.05	5	46.9	5.60	3.0	24.4	21.4	8.60	1.2
10.54	1	50.5	6.02	4.0	29.3	25.3	10.02	1.2
10.55	1	66.5	7.93				11.93	
10.555	1	73.0	8.71				12.71	
10.56	1	74.6	8.83				12.83	
10.565	1	58.5	6.98				10.98	
10.57	1	61.2	7.30				11.30	
10.58	1	63.0	7.52				11.52	
10.59	1	57.0	6.80				10.80	
11.05	5	28.1	3.35	4.2	31.7	27.5	7.55	2.4
12.34	6	18.5	2.20	5.1	32.2	27.1	7.30	2.4
		Average	3.82	2.66			6.48	

The conditions under which the results in Table VI were obtained differ somewhat from those of the preceding Table IV. The air temperature averaged 3.85° C. lower and the average wind velocity was .7 miles per hour greater. Also the difference between the shade temperature and that recorded by the black-bulb thermometer was 2.7° C. lower. Under these conditions the average differential temperature between leaf and air was 3.56° C. and the maximum 5.19° C.

(2) *The Effect of One Fourth to One Half Full Sunlight.*—That a decrease in the intensity of solar radiation, other conditions remaining

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[2] Not included in the averages.

TABLE VI

February 12, 1914

Clear. Moderate breeze. Relative humidity 86.5%

1 Time of Observation	2 No. of Observations	3 Aver. Bridge Reading in Cm.	4 Calculated Temp. Diff.	5 Shade Temp.	6 Black Bulb in Vacuum	7 Difference Columns 5 and 6	8 Actual Leaf Temp.	9 Wind Velocity Miles per Hr.
9.55	1	40.6	5.12°	−19.0°	12.8°	31.8°	−13.88°	1.2
10.20	1	30.0	3.78	−18.1	12.8	30.9	−14.32	1.2
10.48	4	27.8	3.49	−17.2	16.1	33.3	−13.71	2.4
10.58	5	34.2	4.30	−17.0	20.0	37.0	−12.70	1.2
11.05	6	41.4	5.19	−16.0	22.2	38.2	−10.81	1.2
11.46	4	30.0	3.76	−16.0	20.0	36.0	−12.24	2.4
12.26	6	31.8	3.98	−15.8	20.6	36.4	−11.82	1.8
12.36	7	37.0	4.64	−16.2	21.2	37.3	−11.56	1.8
1.38	4	20.3	2.54	−15.5	13.9	29.4	−12.96	1.8
1.47	4	30.7	3.84	−15.0	12.8	27.8	−11.16	1.8
1.56	8	19.3	2.41	−15.2	11.7	26.9	−12.79	1.8
1.36	4	15.4	1.93	−15.0	5.6	20.6	−13.07	1.2
2.50	2	10.5	1.31	−15.2	3.3	18.5	−13.89	1.8
2.54[3]	1	6.5	.81	−15.2	3.3	18.5	−14.39	1.8
		Average	3.56°	−16.25		31.1	−12.68	1.7

constant, is followed by a decrease in the differential temperature between leaf and air needs, of course, no proof. Nevertheless the tables given are of interest since they show the actual temperatures of the leaf under the conditions existing. Results are given in Tables VII, VIII and IX where the column headings are the same for all tables.

TABLE VII

January 16, 1914

Hazy. Light breeze. Relative humidity 93%

1 Time of Observation	2 No. of Observations	3 Aver. Bridge Reading in Cm.	4 Calculated Temp. Diff.	5 Shade Temp.	6 Black Bulb in Vacuum	7 Difference Columns 5 and 6	8 Actual Leaf Temp.	9 Wind Velocity Miles per Hour
11.10	5	21.3	2.54°	5.0°	25.6°	20.6°	7.54°	No data
11.40	6	15.9	1.90	5.2	25.6	20.4	7.10	
11.58	2	15.9	1.90	5.5	25.6	20.1	7.40	
12.30	5	18.2	2.17	5.7	25.6	19.9	7.87	
12.40	6	20.4	2.42	5.8	27.0	21.2	8.22	
12.50	7	26.8	3.18	6.0	27.8	21.8	9.18	
1.10	3	18.9	2.25	6.5	27.5	21.0	8.75	
1.23	7	18.2	2.17	5.3	26.7	21.4	7.47	
1.35[4]	6	8.2	.97	5.2	26.1	20.9	6.17	
		Average	2.17	5.58		20.8	7.74	

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[3] Leaf in shadow; not included in the averages.

[4] Leaf in shadow.

Table VIII

January 25, 1914

Approximately one half full sunshine. Light breeze. Relative humidity 78.5%

1	2	3	4	5	6	7	8	9
9.45	1	22.5	2.77°	−8.5°	16.7°	25.2°	−5.73°	1.2
9.47	1	21.5	2.65				−5.85	
9.48	1	20.3	2.50				−6.00	
9.49	1	20.0	2.46				−6.04	
9.50	1	25.7	3.17				−5.33	
9.53	1	26.8	3.30				−5.20	
11.10	1	26.9	3.31	−8.0	20.0	28.0	−4.69	0.8
11.11	1	33.7	4.14				−3.86	
11.12	1	36.3	4.46				−3.54	
11.15	1	36.3	4.46				−3.54	
11.20	1	32.5	3.99				−4.01	
11.22	1	36.7	4.51				−3.59	
		Average	3.47	−8.25		26.6	−4.78	

Table IX

February 2, 1914

Approximately one fourth full sunshine. Breeze moderately strong. Relative humidity 83.5%

1	2	3	4	5	6	7	8	9
Time of Observation	No. of Observations	Aver. Bridge Reading in Cm.	Calculated Temp. Diff.	Shade Temp.	Black Bulb in Vacuum	Difference Columns 5 and 6	Actual Leaf Temp.	Wind Velocity Miles per Hr.
10.34	1	24.8	3.00°	−1.0°	20.0°	21.0°	2.00°	
10.40	5	14.5	1.75	−0.8	22.2	23.0	.95	4.8
11.00	4	14.0	1.69	−0.5	22.2	22.7	1.19	4.8
11.15	5	11.5	1.39	0	17.8	17.8	1.39	4.8
11.46	5	13.5	1.63	0	18.3	18.3	1.63	4.8
11.55	3	16.1	1.94	0.2	19.2	19.0	2.14	3.7
12.36	3	10.9	1.31	1.0	16.7	15.7	2.31	3.2
1.25	3	18.9	2.27	1.8	19.7	17.9	4.07	3.6
1.31	4	21.5	2.58	2.0	22.2	20.2	4.58	3.6
2.34	3	11.0	1.39	2.5	19.4	16.9	3.89	3.6
		Average	1.89	0.52		19.25	2.41	

With approximately one fourth of full sunlight, and with a light breeze blowing, the maximum differential temperature obtained was 3.18° C. and the average 2.17° C. (Table VII); with approximately the same radiation (compare column 7, Tables VII and IX) and a moderate breeze blowing, the maximum is 3.0° C. and the average only 1.89° C.; with approximately one half of full sunlight (Table VII) and a light breeze, the maximum is 4.51° C. and the average 3.47° C., as compared with a maximum of 7.95° C. and an average of 6.09° C. under full sunlight (Table IV).

(3) *The Effect of Diffuse Light.*—Even on dark, cloudy, winter days, pine leaves absorb sufficient radiant energy to maintain a temperature slightly above that of the air. On a few very dark days the potentiometer gave zero displacement, indicating that the leaf temperature was the same as that of the air, but, as a rule, the leaf was found to be from 0.34° C. to a little more than 2° C. higher than the air temperature, depending upon the brightness of the diffuse light. In only one case was the leaf temperature found to be lower than that of the air during the hours of daylight. This occurred on January 29, when the air temperature was comparatively high. The average for the days given in Table X is 0.95° C.

TABLE X

January 18, 1914

Cloudy. Light breeze. Relative humidity 77%

1	2	3	4	5	6	7	8	9
Time of Observation	No. of Observations	Aver. Bridge Reading in Cm.	Calculated Temp. Diff.	Shade Temp.	Black Bulb in Vacuum	Diff. Columns 5 and 6	Actual Leaf Temp.	Wind Velocity Miles per Hr.
12.30	9	9.6	1.16°	−2.2°	9.4°	11.6°	−1.04°	
12.48	3	8.2	.99	−2.0	7.2	9.2	−1.01	
		Average	1.08	−2.1			−1.02	

January 23, 1914

Cloudy. Breeze moderate. Relative humidity 100%

1	2	3	4	5	6	7	8	9
10.15	5	11.0	1.31°	4.0°	17.8°	13.8°	5.31°	1.2
10.33	6	4.9	.58	5.1	14.4	9.3	5.68	2.4
1.15	5	5.1	.61	6.0	15.6	9.6	6.61	2.4
		Average	.83	5.03			5.86	

February 19, 1914

Cloudy. Breeze moderate. Relative humidity 89.5%

1	2	3	4	5	6	7	8	9
12.38	5	12.9	1.58°	−6.0°	12.8°	18.8°	−4.42°	3.0
1.30	5	17.8	2.17	−5.5	17.2	22.2	−3.33	2.4
		Average	1.88	−5.75			−3.87	

March 22, 1914

Cloudy. Light breeze

1	2	3	4	5	6	7	8	9
8.40	6	2.8	.34°	−0.8°	7.2°	8.0°	−0.46°	
12.30	7	5.6	.67	1.4	9.4	8.0	2.07	
1.30	1	0	0	1.0	2.0	1.0	1.00	
		Average	.34	.53			.87	

(4) *The Leaf Temperature at Night.*—Throughout February and a part of March readings were taken at six and ten p.m. The results obtained are represented by the values given in Table XI. A few readings show no difference in temperature between the leaf and the air, but, as a rule, the leaf is from 0.1° to 0.7° C. colder than the air.

TABLE XI

Date	Bridge Read.		Computed Temp. Diff.		Air Temp.		Rel. Humidity	Atmospheric Conditions
	6 PM.	10 PM.	6 PM.	10 PM.	6 PM.	10 PM.	7 PM.	
Feb. 7	−2.0[5]	−2.5[5]	0.25°	0.32°	−10.0°	−11.0°	80%	Clear; wind light.
" 8	0	−2.0	0	0.25	−18.5	−20.0	67	Clear; wind strong.
" 9	−1.0	−2.5	0.13	0.31	−10.0	− 9.5	74	Clear; wind strong.
" 10	−2.8	0	0.35	0	−12.0	−14.0	55	Wind light 6 P.M.; Calm 10 P.M.
" 11	−2.5	−2.5	0.31	0.32	−16.0	−19.5	77	Wind moderate.
" 12	−2.0	−2.5	0.25	0.32	−17.0	−19.0	73	Wind moderate.
" 13	0	−1.5	0	0.19	−13.0	−14.0	75	Cloudy; wind moderate.
" 16	−1.5	−1.2	0.19	0.15	−10.0	−12.0	81	Cloudy; wind moderate.
" 19	−5.0	−3.6	0.62	0.45	− 9.0	−12.0	88	Clear; calm.
" 25	−3.5	−6.0	0.45	.73	− 7.0	−10.0	44	Clear; calm.
Mar. 5	−1.0	−1.5	0.12	0.18	0	− 2.0	78	Cloudy; wind light.
" 6	−0.5	0	0.06	0	0.5	0	73	Cloudy; wind light.
" 7	−0.3	0	0.04	0	− 1.5	− 4.0	100	Snowing; wind light.
		Average	0.21	0.25				

(5) *Effect of the Wind in Reducing the Leaf Temperature.*—The effect of the wind in reducing the leaf temperature is well illustrated in Tables XII and XIII. The average differential temperature between leaf and air for the two tables is 2.93° C. as compared with 6.04° C. in Table IV. A part of this reduction in differential tempera-

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[5] Negative sign under bridge reading indicates that the current was reversed and that the leaf was colder than the air.

ture is due, no doubt, to a difference in intensity of insolation, column 7 of Table IV showing slightly higher values than the corresponding columns in Tables VI, XII and XIII. But the difference in the intensity of insolation is slight and can account for only a small part of the reduction. The wind, therefore, is the important factor.

TABLE XII

February 1, 1914

Clear. Strong wind.[6] Relative humidity 71%

1	2	3	4	5	6	7	8	9
Time of Obser-vation	No. of Obser vations	Aver. Bridge Readings in Cm.	Computed Temp. Diff.	Shade Temp.	Black Bulb in Vacuum	Diff, Columns 5 and 6	Actual Leaf Temp.	Wind Velocity Miles per Hr.
12.35	3	17.4	2.11°	−3.0°	28.6°	31.6°	−0.89°	6.0
12.45	6	22.1	2.68	−3.1	29.0	32.1	−0.42	6.0
1.50	11	27.0	3.28	−3.5	29.2	32.7	−0.22	6.0
1.58	5	23.8	2.89	−3.2	20.0	23.2	−0.31	4.8
		Average	2.74	−3.2		29.9	−0.46	

March 2, 1914

Relative humidity of 90%

1	2	3	4	5	6	7	8	9
8.52	7	15.4	1.91°	−12.2°	9.4°	21.6°	−10.29°	4.8
9.36	4	17.1	2.12	−11.3	21.1	32.4	− 9.18	4.8
10.06	8	23.0	3.83	− 8.5	27.2	35.7	− 4.67	5.0
11.00	1	20.0	2.46	"	"	"	− 6.04	3.6
	1	10.5	1.29	"	"	"	− 7.21	"
	1	7.5	.92	"	"	"	− 7.58	"
	1	22.6	2.78	"	"	"	− 5.72	"
	1	26.5	3.26	"	"	"	− 5.24	"
	1	13.2	1.62	"	"	"	− 6.88	"
	1	19.0	2.34	"	"	"	− 6.16	"
	1	7.0	.86	"	"	"	− 7.64	"
	1	22.5	2.77	"	"	"	− 5.73	"
	1	39.3	4.83	"	"	"	− 3.67	"
11.05	1	28.0	3.44	"	"	"	− 5.06	"
12.31	8	16.8	2.06	− 6.5	26.1	32.6	− 4.44	4.8
		Average	2.61	− 9.4		31.6	− 6.93	

The effect of gusts of wind is shown in the rapid fluctuations in the differential temperature for the period 11.00 to 11.05, Tables IV and XIII. Fluctuations of 2° and 3° C. occur within one half minute.

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[6] Wind velocity, University Observatory record, Feb. 1, 15 miles per hour; March 2, 26 miles per hour.

TABLE XIII

February 8, 1914

Clear. Strong wind.[7] Relative humidity 71%

1	2	3	4	5	6	7	8	9
Time of Observation	No. of Observations	Aver. Bridge Reading in Cm.	Calculated Temp. Diff.	Shade Temp.	Black Bulb in Vacuum	Diff. Columns 5 and 6	Actual Leaf Temp.	Wind Velocity Miles per Hr.
9.45	2	16.7	2.09°	−15.5°	10.0°	25.5°	−13.41°	6.0
10.15	1	20.2	2.48	−14.5	11.1	25.6	−12.02	6.0
10.37	7	24.0	3.00	−14.5	15.6	30.1	−11.50	6.0
10.47	7	23.1	2.87	−14.5	17.2	31.7	−11.63	6.0
10.56	5	30.1	3.75	−14.3	18.2	32.5	−10.55	4.8
11.04	6	34.5	4.30	−14.0	19.8	33.8	− 9.70	6.0
11.45	7	32.8	4.09	−14.2	19.9	34.1	−10.11	6.0
12.17	12	38.7	4.83	−13.8	22.2	36.0	− 8.97	6.0
1.38[8]	8	28.7	3.58	−13.8	22.2	36.0	−10.22	6.0
		Average	3.44	−14.34		31.7	−10.90	

These fluctuations by no means represent all the changes in the leaf temperature. In fact, the changes were so rapid that it was not possible to adjust the sliding contact quickly enough to record them all. A somewhat nearer approach toward recording all the fluctuations was made in the following way: The galvanometer was calibrated at the existing temperature in terms of displacement along the bridge wire. The sliding contact was held at a fixed point, and the galvanometer deflections read every five seconds for a period of two minutes, an assistant indicating the periods and recording the deflections as read. To insure that the deflections should be due solely to changes in the leaf temperature, the second junction was enclosed in a thermos-bottle and kept at a constant temperature. The results are graphically represented by figure 4.

One series of observations and measurements was made on a detached branch with cut end immersed in water, and the preparation protected from the wind by a thin cloth screen except on the south side. The results, given in the following table, show that in quiet air the temperature of the leaf may rise to 10° above that of the surrounding air.

In making up the averages the average readings for the periods are taken.

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[7] Wind velocity, university observatory record, 18 miles per hour.

[8] Leaf in shadow for a part of the period.

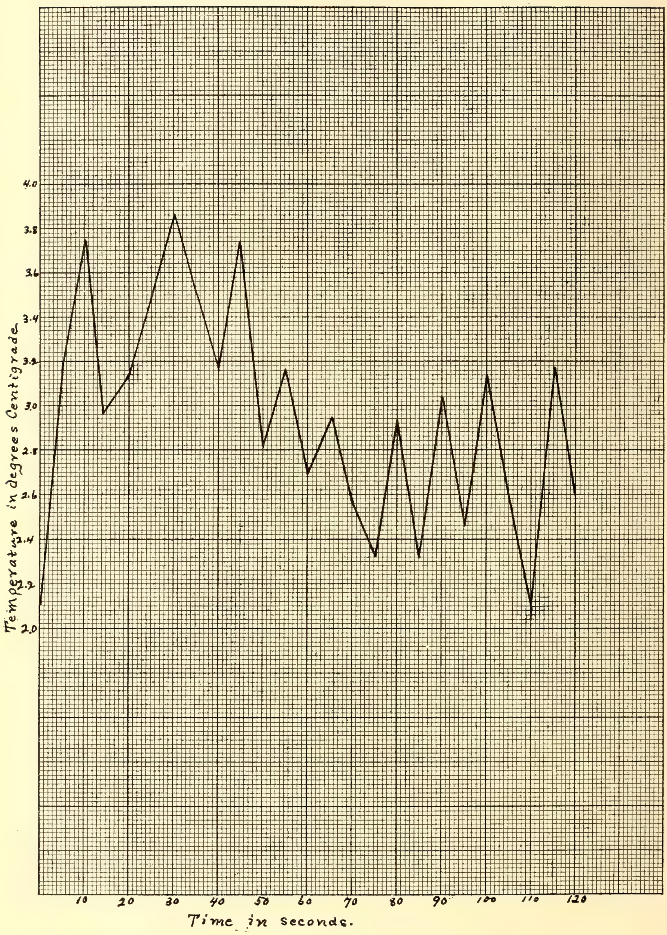

FIG. 4. Graph showing galvanometer deflections due to changes in leaf temperature.

TABLE XIV

March 10, 1914

Clear. Breeze light. Relative humidity 76%

Detached branch partially protected against air currents by cheese cloth screen.

1	2	3	4	5	6		8
Time of Observation	No. of Observations	Aver. Bridge Reading in Cm.	Calculated Temp. Diff.	Shade Temp.	Black Bulb in Vacuum	Diff. Columns 5 and 6	Actual Leaf Temp.
10.45	1	64.0	7.69°	2.0°	32.2°	30.2°	9.69°
	1	50.5	6.07				8.07
10.46	1	63.8	7.67				9.67
10.47	1	73.0	8.77				10.77
10.48	1	85.8	10.31				12.31
10.49	1	82.0	9.86				11.86
10.50	1	75.0	9.02				11.02
11.47	1	74.0	8.83	4.2	35.0	30.8	13.03
	1	71.5	8.53				12.73
11.48	1	61.0	7.28				11.48
	1	77.0	9.17				13.37
	1	74.2	8.85				13.05
11.50	1	79.4	9.47				13.67
	1	79.4	9.47				13.67
	1	73.0	8.71				12.91
11.51	1	74.4	8.88				13.08
12.40	1	75.5	8.98	5.5	36.7	31.2	14.48
	1	80.0	9.52				15.02
12.41	1	63.5	7.56				13.06
	1	66.0	7.85				13.35
12.42	1	71.5	8.51				14.01
	1	69.5	8.27				13.77
12.43	1	76.4	9.09				14.59
	1	65.0	7.73				13.23
12.44	1	75.0	8.93				14.43
	1	61.8	7.35				12.85
12.45	1	76.5	9.10				14.60
12.54	1	82.0	9.76	5.5	36.7	31.2	15.26
12.55	1	85.2	10.12				15.62
1.05	1	83.0	9.88	5.50	35.0	30.0	14.88
	1	64.5	7.68				12.68
1.06	1	79.5	9.46				14.46
1.07	1	72.5	8.63				13.63
		Average	8.91	4.44			13.35

(6) *The Temperature of Leaves at Different Angles to the Sun's Rays.*—Finally an experiment was made to determine the difference in temperature between two leaves at different angles to the sun's

rays—one at approximately 45°, the other at 90°. For this purpose a cut branch, partially protected from the wind by a screen of white cheese cloth, was exposed to solar illumination. The screen was open at the top to prevent the enclosed air from becoming heated. Doubtless there was some reflected radiation, but, since the leaves were side by side within the enclosure and therefore under the same conditions, this did not affect the results. These are given in Table XV. Other attempts in which the differential temperatures of the two leaves were read alternately in rapid succession gave similar results.

TABLE XV

March 5, 1914

Temperature of leaves at different angles

	Time of Observation	Aver. Bridge Reading in Cm.	Calculated Temp. Diff.	Shade Temp.	Black Bulb in Vacuum
Angle 45°	2.15	45.4 49.8 46.3 48.0 38.6 43.0 31.0	5.29° 5.80 5.39 5.59 4.50 5.01 3.61	14.0°	36.1°
	2.20	35.5	4.14		
	Average	42.2	4.92		
Angle 90°	2.25	58.9 64.2 64.5 57.1 49.0 47.0 54.5 50.0	6.86° 7.47 7.51 6.65 5.71 5.48 6.35 5.83	14.2°	36.0°
	2.30	54.6	5.36		
	Average	55.5	6.36		

B. *Direct Evidence of Photosynthesis in Winter*

An attempt was made to obtain direct evidence of photosynthesis under winter conditions by examining for starch content the leaves of the various conifers growing in the university arboretum. The leaves were collected in early morning and again in late afternoon of clear days. They were kept in 75 per cent alcohol and later examined microscopically. Thin sections were placed on a slide and treated with absolute alcohol and ether to remove the fats, and then with a solution of chloral-iodine and potassium iodide. During the autumn and through December the leaves of *Pinus laricio austriaca* Endl. only were examined. Later other species were added. From five to ten leaves were examined in each case.

In the leaves of *Pinus laricio austriaca* starch remained abundant in mesophyll, endodermis, and transfusion tissue throughout October and November. But leaves collected December 2, after a period of seven dark, rainy days—maximum temperature for the entire period 14° C. and the mean 6.7° C.—showed, when examined, no starch in the mesophyll and only a small amount in the endodermis. Leaves collected from the same branch December 4—maximum temperature 11.6° C. and the mean 6.7° C.—showed a small amount of starch in the mesophyll, endodermis, and transfusion tissue. On December 11, the temperature, in the meantime, having dropped to a minimum of − 7° C. and a maximum of 3° C., the starch had entirely disappeared from all the tissues. Starch was again found on December 12 in the mesophyll, endodermis, and transfusion tissue, and in greater abundance on the following day, the temperature having risen to a maximum of 12 ° C.

Since the temperature on December 4 was colder by several degrees than that of the preceding days, the reappearance of starch could not be due to a regeneration from sugar already present in the leaf, but must have been the result of photosynthesis. On December 13, however, the maximum temperature had risen 9° C. above that of December 11, and the reappearance of starch may have been the result, in part at least, of a regeneration of starch from the sugar present in the leaf, due to an increase in temperature.

When next examined, December 20, starch had entirely disappeared from the mesophyll, though a small amount still remained in the endodermis and in the transfusion tissue. By January 15 the leaves were entirely free from starch with the exception of an occasional group of hyaline mesophyll cells, where it was evidently stored.

Throughout January, February, and early March leaves of a number of conifers were collected and examined, but no further conclusive evidence of photosynthesis was obtained. Some of the species examined retained starch in the mesophyll in greater or less abundance throughout the winter, but no increase in quantity, even after a comparatively warm bright day, could be detected microscopically. In other species groups of hyaline cells filled with large starch granules were observed more or less regularly. These groups usually appeared immediately below the epidermis. No explanation is attempted. The author hopes to make this a subject for further investigation.

While no conclusive evidence of starch formation was observed after December 13, it does not follow that all photosynthetic activity had ceased. Carbohydrates may have been formed and used or translocated as fast as formed. It merely indicates that photosynthesis was not sufficiently active under the conditions that obtained during January and February to result in the production of starch in the leaf.

The species examined and the results obtained are as given below.

No starch: *Pinus strobus* L.

More or less starch:

Juniperus communis L.
Juniperus virginiana L.
Tsuga canadensis (L.) Carr.
Thuya occidentalis L.
Picea Abies (L.) Karst.
Abies balsamea (L.) Mill.

Starch in groups of hyaline cells:

Pinus laricio austriaca Endl.
Pinus sylvestris L.
Pinus ponderosa Laws.
Pinus Banksiana Lamb.

These results do not agree with those of Schulz (26) and Lidforss (13). The former maintains that the leaves of all gymnosperms except the Gnetaceae contain no starch in winter; the latter states as a universal rule that all green plant cells are free from starch during the winter months. Evidently their results do not hold for southern Michigan just as they do not hold for the warmer portions of Japan as was shown by Miyaké (18). This problem should be given more extended investigation.

V. SUMMARY

The data presented above show that evergreen conifer leaves, even under winter conditions, through the absorption of radiant energy, maintain temperatures from 2° to 10° C. higher than the surrounding air. The maximum obtained under brilliant illumination and with a light breeze blowing was 8.83° C. (Table V). In still air the temperature difference is considerably higher. This was shown by an experiment in which the leaf was partially protected against air currents. Under these conditions a differential temperature of 10.31° C. was obtained (Table XIV). Under less brilliant illumination and stronger air currents the differential temperature is correspondingly less. Even diffuse light, according to its brightness, will increase the leaf temperature from 0.5° to 2° C. For February, the coldest month of the year, the average differential temperature between leaf and air—650 readings in all, taken between the hours of 8 a.m. and 3 p.m. and under all kinds of weather conditions, cloudy days as well as days of sunshine—was 3.06° C.

The differential temperatures in the winter season, as was to be expected, are considerably less than those obtained by previous investigators for broad leaves under summer and tropical insolation, namely, from 7° to 16° C. The cause of the difference is to be sought in the lower rate of respiratory changes (Molisch (19)), the lower intensity of solar radiation, and in the greater loss of heat by convection, air currents being, as a rule, more constant and stronger during the winter season. Though smaller in winter, the differences are, nevertheless, of sufficient magnitude to become an important factor in photosynthesis.

It should also be remembered that the differential temperatures as found in this investigation are those of the leaf as a whole, and not necessarily those of the chloroplasts. It is the chloroplasts that absorb the most of the radiant energy; the temperature of the leaf is due to radiation and conduction from the chloroplasts. It is entirely probable, therefore, that the chloroplasts have a temperature considerably higher than that of the leaf as a whole.

Recalling briefly the more important facts of the evidence at our disposal concerning photosynthesis at low temperatures, we have:

(1) Evidence of an accumulation of reserve food material through the winter in trees with persistent leaves as found by Sablon; (2) confirmatory evidence both of photosynthesis and of an accumulation of reserve food in winter by evergreen leaves as found by Miyaké; (3) evidence of photosynthesis at − 6° C. as the result of very careful work by Matthaei; (4) the finding of Ewart that a number of evergreen shrubs and trees including conifers, after having been exposed for some time to a temperature often falling below − 15° C. had no power of assimilation when tested at 1° C.

The results obtained by Ewart appear to contradict those of

Miyaké and Matthaei. In making comparisons it should be borne in mind, however, that Matthaei's material had previously been kept at a uniform temperature from 10° to 16° C., and that Miyaké's experiments were made at Tokyo where the mean temperature for the three winter months is 3.8° C. and the minimum − 6.5 (Kusano (12)), while the plants from which Ewart obtained his material had been exposed for three weeks to temperatures falling below − 15°C. Ewart ascribes the inability of these plants to assimilate CO_2 at 1° C. to the inhibiting effect of the extreme temperature to which they had been exposed. The same plant material, when brought to a temperature of 15° C., showed weak assimilation within a few hours and quite active assimilation within eight hours to one day. The long latent period of recovery, according to Ewart, indicates that the exposure had nearly reached the plant's limit of resistance. Unfortunately Ewart does not state how long the tests for assimilation at 1° C. lasted. One gains the impression that the tests were of short duration. It is entirely possible that, had the plants been exposed to a temperature of 1° C. for a longer time, the inhibiting effect of the previous extreme temperature would have been overcome and assimilation begun. The contradiction between Ewart's results on the one hand and those of Matthaei and Miyaké on the other is, therefore, more apparent than real.

Bearing in mind the assimilation curve obtained by Matthaei, the application of the results of the present investigation becomes at once apparent. Kanitz (10) has shown that the law of van't Hoff (32), that for every rise of 10° C. the rate of reaction is doubled or trebled, holds for the results obtained by Matthaei for temperatures between 0° and 37° C. assuming other conditions favorable. For temperatures from − 6° to 0° C. the rate of increase is very much greater. The assimilation curve in Matthaei's work shows an increase in assimilation from 2 mmg. of CO_2 per 50 sq. cm. per hour at − 6° C., to 18 mmg. at 0° C.,—an increase of 900 per cent. Since an increase of 6° C. in the temperature of the leaf due to the absorption of radiant energy is not at all uncommon and higher temperature differences are often found, the increase in assimilation resulting therefrom is of very great importance and goes far toward explaining the accumulation of reserve food material by evergreen trees.

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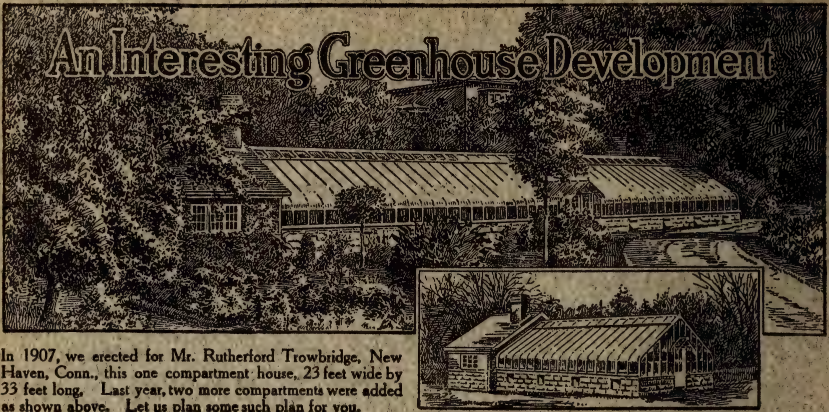

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SOME EFFECTS OF THE BROWN-ROT FUNGUS UPON THE COMPOSITION OF THE PEACH[1]

LON A. HAWKINS

The morphology and life-history of *Sclerotinia cinerea* (Bon.) Schröter, the fungus causing brown-rot of the peach, have been investigated with considerable care. The fact that this fungus is parasitic upon a number of other hosts is also well established. Not so much attention, however, has been paid to the effect of the fungus upon the composition of the peach; and it was to obtain some information upon this subject that the experiments described in this paper were carried out.[2]

In these experiments most of the peaches were inoculated in the laboratory, though one series of analyses was made using peaches which had become infected under orchard conditions. The inoculations were made from stock cultures of the fungus grown on potato agar. The fungus was originally isolated from fruit in the early stages of brown-rot by removing portions of the deeper-lying tissue and transferring them to tubes of sterile potato agar. Pure cultures were obtained by this method and the fungus maintained in stock culture throughout the investigation.

The method for studying the effect of the fungus upon the tissue of the peach was somewhat similar to that used by Behrens (1) in

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[2] The writer's thanks are due to Mr. J. W. Kelly, laboratory technician in this office, for assistance in the experimental part of this work.

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studying the effects of *Sclerotinia fructigena* (Pers.) Nort. upon apples. This writer made a single inoculation upon the side of each apple. When the fruit was half rotted he split the rotten half from the sound portion and analyzed the two parts separately.

In the present investigation this method was considerably modified. The peaches were first divided into quarters and the opposite quarters were combined. One of the two samples thus obtained was inoculated and the other was used as a control. Before the peaches were sampled, however, they were thoroughly scrubbed with a solution of bichlorid of mercury 1 to 1,000. They were quartered and separated from the stone with a sterile knife and immediately placed in sterile, glass-stoppered weighing bottles which had been tared, and then weighed. One set of these samples consisting of one sample from each peach was inoculated from the stock cultures of the fungus by shaking up spores in sterile water and pouring them over the cut surfaces of the fruit. The other set, the controls, were treated with a like quantity of sterile water. The two sets of samples were kept side by side under the same temperature and moisture conditions for two weeks or more. At the end of this time the samples were examined and any of the control samples found to be infected with fungi were discarded together with the corresponding inoculated halves. This procedure was also followed with any of the inoculated portions found to be infected with fungi other than Sclerotinia. As a further precaution cultures on beef agar were made from the interior of the remaining samples and if any of these cultures indicated the presence of contaminating fungi or bacteria in the fruit the samples were discarded. After these inoculations were made the samples were prepared for analysis.

In the analyses, determinations were made of the pentosan, acid, and sugar content of the peach samples. The amount of alcohol-insoluble material which reduced Fehling's solution when hydrolyzed with dilute hydrochloric acid, was also determined. The data thus obtained were calculated on the basis of wet weight of fruit at the time of inoculation and it was then possible to compare the content of the different compounds in the sound and rotten portions of the same peach.

In the pentosan and acid determinations it was found advisable to use an entire sample for each determination. In the case of the sugars, however, a sample served for the two sugar determinations and for the determination of alcohol-insoluble substance which reduced

Fehling's solution upon hydrolysis with dilute hydrochloric acid. The samples were prepared for analysis by slicing as thinly as possible with a sharp knife and then chopping up the slices. Inasmuch as all determinations were related to original wet weight, care was taken that none of the pulp or juice was lost.

For pentosan determinations the chopped-up peach pulp was washed directly into half liter Erlenmeyer flasks and distilled immediately. Pentosan determinations were made according to Tollens's phloroglucid method (8) and the phloroglucid was calculated as pentosan according to Krober's (8) tables. Methyl-pentosan determinations made on portions of a number of peaches according to Ellett and Tollens's (9) method, showed the presence in the peach-pulp of substances yielding methyl-furfurol when boiled with hydrochloric acid. The amount, however, was so small that it was not considered worth while to determine it in all the samples.

The samples for acid determination were washed into 250 cc. Erlenmeyer flasks with 100 cc. of water and heated nearly to boiling for one hour. The mixture was then transferred to 250 cc. volumetric flasks which were filled to volume with water and allowed to stand with frequent shaking for one week. Toluol was added to prevent the action of micro-organisms. At the end of a week the solutions in the flasks were again made up to volume, filtered, and two 50 cc. portions of the filtrate titrated against standard potassium hydroxide. The acidity of the samples was calculated from these data in cc. normal acid per 100 g. original wet weight.

The samples in which the sugar determinations were made were washed into 250 cc. volumetric flasks with 70 per cent alcohol. About a gram of calcium carbonate was added to neutralize the acidity. The flasks were filled up to volume with alcohol and allowed to stand with frequent shakings for one week. At the end of this time the solutions were again made up to volume, filtered, and 200 cc. of the filtrate pipetted into beakers. The alcohol was driven off and the residue washed into volumetric flasks with water. The solution was cleared with neutral lead acetate, made up to the original volume and filtered. The excess lead was precipitated as oxalate, by adding sodium oxalate. The solution was again filtered and the amount of reducing sugar determined in the filtrate, using Allihn's modification of Fehling's solution (8). The copper was determined by direct weighing of the cuprous oxide and the dextrose calculated according to

Allihn's tables. The method used for sugars was similar to that given by Bryan, Given and Straughn (10).

The total sugars were determined in the solution used for reducing sugars by inverting the sucrose in 50 cc. with hydrochloric acid, making the solution up to 100 c.c. and neutralizing with anhydrous sodium carbonate. The sugar was determined as in the case of the reducing sugar. The sucrose content was calculated from the difference in total and invert sugar in the sample, as is the usual procedure in investigations of this kind. Kulisch (7), Girard (5), Bigelow and Gore (2) and others have considered this difference between the total and reducing sugars in peaches to be due to sucrose. It was, however, considered worth while, in view of the work of Davis and Daish (4), to obtain more evidence upon this point. Accordingly, a number of peaches were sliced up, a little calcium carbonate added, and the mixture extracted with 70 per cent alcohol, for one week. The solution was then filtered and three samples of the filtrate measured out. These solutions were prepared for analysis in the same way as the solutions from samples of peaches in the inoculation experiments and the reducing sugars determined. For total sugars duplicate samples were pipetted from each of the three solutions. The one set of these was treated with acid in the usual way, while the sugar in the other set was inverted with invertase.[3] The percentage of reducing sugar in the solutions and the percentage of total sugar as found by both methods are given below:

	Per Cent Sugar as Glucose after Inversion (Total Sugar)	
Per Cent Reducing Sugar	Acid Inversion	Invertase Inversion
0.77	3.34	3.37
0.77	3.36	3.48
0.77	3.47	3.46

From these results it would seem probable that the increase in reducing substance after treatment with acids is due to the inversion of the cane-sugar in the solution. At any rate, according to the work of Hudson (6), the increase in reducing power is not due to the hydrolysis of starch, dextrins or pentosans or to the inversion of maltose or lactose. The alcoholic sugar solution, from which the original samples were taken, was later cleared with lead acetate, filtered, and the excess lead removed as sulphide. The filtrate was evaporated and a sugar crystallized out. After several recrystallizations this sugar was identified as sucrose by its melting point, by the fact that it did not reduce Fehling's solution until after inversion with acid or invertase and by its specific rotation.

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[3] The writer's thanks are due Dr. C. S. Hudson, of the Bureau of Chemistry, for the invertase solution used in these experiments.

As has been said, the alcoholic sugar solution was filtered off the peach sample and the amount of sugar determined in the filtrate. The residue on the filter was carefully removed to a porcelain extraction thimble and extracted for one day continuously in a Soxhlet extractor. It was then dried, ground up and treated according to the usual routine for the determination of starch. The determinations were made by the direct acid hydrolysis method (8), as in the case of the sugars. Whether any considerable amount of these substances, which are insoluble in alcohol and reduce Fehling's solution after hydrolysis with dilute hydrochloric acid, are starch, is an open question. Bigelow and Gore (2) found starch grains in young peaches located only in a thin layer just under the epidermis. Determined quantitatively they found only one tenth of one per cent. The present writer examined portions of the ground pulp microscopically and, while bodies were present which gave the blue color with iodine, no definitely formed starch grains were discovered.

To obtain more evidence upon this point, six twenty-five gram samples of peach pulp were weighed out and extracted. After drying, three of the samples were treated by the direct acid hydrolysis method and the reducing substances determined in the usual way. The other three samples were carefully ground with fine sand and then digested with malt diastase and treated as in the diastase method for the determination of starch (8). The reducing substance was determined as in the other three samples. A comparison of the percentage of reducing substance in the two sets of samples, calculated as starch, is given below.

Acid Hydrolysis	Diastase Method
1.24	0.32
1.33	0.38
1.34	0.37

From the results of these determinations it seems that a portion of alcohol-insoluble substance which reduces Fehling's solution after hydrolysis with dilute hydrochloric acid, is either starch or some compound, such as dextrin, which is liquefied by diastase. The present work and the investigations of Bigelow and Gore would seem to indicate that in the peach fruit starch is not a common form of reserve for carbohydrates translocated from other parts of the plant.

The method of studying the effect of the fungus upon the peach in this work was to compare the percentage of the compounds as determined in the two samples taken from the same peach, one of which had been inoculated with the fungus, while the other remained sound. Any considerable variation between the two halves in the percentage of the substances determined was considered to have been caused by the fungus. This method is based on the assumption that the content of any of the substances determined is the same in the two samples of the same peach. It seemed then, of some importance to determine the extent of the variation between the two halves of the peach sampled in this way. Accordingly, a series of experiments were carried out using sound peaches and following the same method of sampling as in the inoculation experiments, except that the halves were prepared for analysis immediately after sampling. The same variety of peaches, namely Champion, was used in these determinations as in the inoculation experiments described later.

The results of these analyses are given in Tables I and II, which follow:

TABLE I

PENTOSAN AND ACID CONTENT, AND THE AMOUNT OF ALCOHOL-INSOLUBLE SUBSTANCE WHICH REDUCES FEHLING'S SOLUTION WHEN HYDROLYZED WITH DILUTE HCl, IN SOUND PEACHES, EACH SUBSTANCE DETERMINED IN THE TWO HALVES OF THE SAME PEACH

	Per Cent of Pentosans, Wet Weight		Acid Content in cc. Normal Acid per 100 g., Wet Weight		Per Cent Alc.-Insol. Substance (as Starch) Reducing Fehling's Sol. when Hydrolyzed with Dil. HCl, Wet Weight	
	Half *a*	Half *b*	Half *a*	Half *b*	Half *a*	Half *b*
1	1.21	1.28	13.55	13.21	1.92	1.76
2	1.26	1.37	11.57	12.50	1.54	1.48
3	1.03	1.05	14.67	15.64	1.78	1.64
4	0.97	0.97			1.92	2.01
5					2.01	1.88

From the results in the foregoing tables it seems that there is occasionally some variation in the composition of the two samples. This variation, however, is not as great as between the individual peaches.

Two series of experiments were carried out in which the peaches were inoculated in the laboratory. In the first series of experiments

TABLE II

REDUCING SUGAR, TOTAL SUGAR, AND SUCROSE CONTENT IN THE HALVES OF THE SOUND PEACHES, STATED AS PERCENTAGE OF WET WEIGHT

	Reducing Sugar as Glucose		Total Sugar as Glucose		Sucrose	
	Half *a*	Half *b*	Half *a*	Half *b*	Half *a*	Half *b*
1	4.23	4.33	7.14	6.63	2.57	2.17
2	4.01	4.24	6.00	6.28	1.98	1.91
3	3.50	3.54	5.52	5.56	1.92	1.92

twenty peaches were picked, sampled and inoculated July 23. They were prepared and inoculated as already described and were allowed to remain in the glass-stoppered weighing bottles which were placed in a large covered glass dish until August 11. They were then examined and prepared for analysis.

The second series of experiments was set up August 14; the same number of peaches were used as in the first series and they were treated in the same way. The samples were prepared for analysis in the usual way August 30. The results of the determinations in the two series of inoculation experiments are given in tables 3–6, which follow:

TABLE III

PENTOSAN AND ACID CONTENT AND THE CONTENT OF ALCOHOL-INSOLUBLE SUBSTANCE WHICH REDUCES FEHLING'S SOLUTION WHEN HYDROLYZED WITH DILUTE HCl, IN THE SOUND AND ROTTEN HALVES OF PEACHES, EACH SUBSTANCE DETERMINED IN SOUND AND ROTTEN HALVES OF THE SAME PEACH

	Per Cent of Pentosans, Wet Weight		Acid Content in cc. Normal Acid per 100 g., Wet Weight		Per Cent Alc.-Insol. Substance (as Starch) Reducing Fehling's Sol. when Hydrolyzed with Dilute HCl, (Wet Weight)	
	Sound Half	Rotten Half	Sound Half	Rotten Half	Sound Half	Rotten Half
1	0.93	0.92	4.75	5.70	1.54	1.39
2	1.17	1.17	7.54	8.85	1.12	1.05
3	0.88	0.94	4.28	5.32	1.30	0.95

TABLE IV

REDUCING SUGAR, TOTAL SUGAR, AND SUCROSE CONTENT IN THE SOUND AND ROTTEN HALVES OF PEACHES, STATED AS PERCENTAGE OF WET WEIGHT

	Reducing Sugar, as Glucose		Total Sugar, as Glucose		Sucrose	
	Sound Half	Rotten Half	Sound Half	Rotten Half	Sound Half	Rotten Half
1	2.41	2.83	4.43	2.836	1.92	0.004
2	2.06	3.08	4.59	3.24	2.31	0.14

TABLE V

PENTOSAN AND ACID CONTENT, AND THE AMOUNT OF ALCOHOL-INSOLUBLE SUBSTANCE WHICH REDUCES FEHLING'S SOLUTION WHEN HYDROLYZED WITH DILUTE HCl, IN THE SOUND AND ROTTEN HALVES OF PEACHES. EACH SUBSTANCE DETERMINED IN SOUND AND ROTTEN HALVES OF THE SAME PEACH.

	Per Cent Pentosans Wet Weight		Acid Content in cc. Normal Acid per 100 g., Wet Weight		Per Cent Alc.-Insol. Substance (as Starch) Reducing Fehling's Sol. When Hydrolyzed With Dil. HCl, Wet Weight	
	Sound Peach	Rotten Peach	Sound Peach	Rotten Peach	Sound Peach	Rotten Peach
1	0.57	0.59	2.45	7.40	0.83	0.80
2	0.67	0.69	4.75	7.30	0.73	0.64
3	0.62	0.66	4.25	6.55	0.71	0.67
4					0.85	0.59

TABLE VI

REDUCING SUGAR, TOTAL SUGAR, AND SUCROSE CONTENT IN THE SOUND AND ROTTEN HALVES OF PEACHES, STATED AS PERCENTAGE OF WET WEIGHT.

	Reducing Sugar as Glucose		Total Sugar as Glucose		Sucrose	
	Sound Half	Rotten Half	Sound Half	Rotten Half	Sound Half	Rotten Half
1	2.47	4.14	5.95	4.26	3.31	0.12
2	1.35	1.68	3.93	1.88	2.44	0.19
3	1.14	2.63	3.03	2.82	1.79	0.18

A third series of analyses was begun August 16, using fruit that had become infected under orchard conditions. A quantity of peaches were picked, some of which bore typical brown-rot spots upon the surface. A number of the peaches which were apparently sound were selected, the pulp removed from the stones and cut up into thin slices. The whole mass was then chopped up into small pieces, well mixed and sixteen twenty-five gram samples weighed out, four for the determination of each of the various substances. In addition to these, three ten-gram samples were weighed out for the determination of the total dry matter. These last were covered with 95 per cent alcohol in glass-stoppered weighing bottles and the samples allowed to stand for several days. Most of the alcohol and water was then driven off at about 60° C. The residue was dried to constant weight in an oven at a temperature of 100° C. and the amount of dry matter calculated.

The infected peaches were allowed to remain under warm humid conditions for two days after the sound peaches were sampled. At the end of this time they were rotten and in most cases covered with tufts of conidiophores of the fungus. They were sliced up and sampled and the samples treated as in the case of the sound peaches. The results of the determinations are given in Table VII, which follows.

Table VII

Pentosan, Acid and Sugar Content and Amount of Alcohol-Insoluble Substance Which Reduces Fehling's Solution on Hydrolysis with Dilute HCl, in Sound and Rotten Peaches from Same Tree

	Wet Weight		Dry Weight	
	Sound	Rotten	Sound	Rotten
Per cent dry matter	14.40	14.40		
Per cent reducing sugar	4.28	8.50	29.75	59.05
Per cent total sugar	9.32	8.59	64.69	59.65
Per cent sucrose	4.89	0.08	33.22	0.57
Per cent alc. insol. substance (as starch) which reduces Fehling's sol. when hydrolyzed with dil. HCl	1.30	1.07	9.05	7.44
Per cent pentosans	0.76	0.73	5.30	5.08
Cc. normal acid per 100 g.	10.53	13.00	73.12	90.28

From the results in the foregoing tables it is evident that some of the compounds studied are much more readily available for the metabolism of the fungus than others.

The pentosan content of the sound and rotten halves of the peaches was usually about the same, the differences being no greater than the variations in pentosan content of the two halves of a sound peach. Moreover, the pentosan content was sometimes higher in the rotten half of the peach than in the corresponding sound half. It seems probable then, that the pentosans were not utilized by the fungus. Cooley (3) has recently shown that the hyphae of this fungus are not found to any considerable extent in the middle lamellae of the cells and it does not apparently digest the pectin of plum fruits. It is possible, though not probable, that part of the pentosan, or furfurol-yielding material, might be used by the fungus and a like amount of pentosans laid down.

The acid content was always higher in the rotten half of the peach than in the sound portion and this difference was greater than the variation in acid content between the two halves of a sound peach. It would seem then, that the fungus forms some acid or causes it to be formed by the peach. No attempt was made in the present investigation to identify the acids of either the sound or rotten peach. This work would, however, seem to corroborate the investigations of Cooley (3) who found that oxalic acid was formed when this fungus was grown upon peach-juice. Behrens (1), on the other hand, found in his work with apples that the acid content of the rotten half of the apple was considerably less than that of the sound portion. In this case the fungus apparently used the acid.

The percentage of alcohol-insoluble substance which reduces Fehling's solution when hydrolyzed with dilute hydrochloric acid was somewhat less in all the rotten samples than in the corresponding sound halves. It is, therefore, to be concluded that the action of the fungus tends to decrease the amount of this substance in the peach, although the variation between the sound and rotten halves of the same peach is occasionally less than that found between the two halves of a sound peach, as shown in Table I, columns 6 and 7. The substance may be utilized by the fungus or changed so that it is soluble in alcohol. In the latter case it would have been extracted with the filtrate used in the determination of the sugars.

In sugar content the sound and rotten halves of the peaches varied considerably. There was more reducing sugar in the rotten halves than in the corresponding sound portions, while in the total sugar content the order was reversed. There was very little cane-sugar in the rotten portions, much less than in the sound samples. The fungus then uses the sugar and causes the inversion of the sucrose. That the sucrose is inverted much more rapidly than the invert sugar is used by the fungus is evident from the fact that the reducing sugar content is higher in the rotten samples. This is especially evident in the case of the peaches rotted after they had become infected under orchard conditions in which only a small amount of the sugar had been used yet nearly all the cane-sugar had been inverted. Behrens (1) found that *Sclerotinia fructigena* used the sugar in apples; he, however, measured only the total sugars.

In conclusion, it may be said that in peaches rotted by the brown-rot fungus, *Sclerotinia cinerea*, the pentosan content remains practically the same, the acid content is increased, the amount of alcohol-insoluble substance which reduces Fehling's solution when hydrolyzed with dilute hydrochloric acid decreases, the total sugar content decreases, while the cane-sugar practically disappears.

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NEGATIVE HELIOTROPISM OF UREDINIOSPORE GERM-TUBES[1]

F. D. Fromme

During the course of some germination tests with urediniospores of *Puccinia Rhamni* (Pers.) Wettst. in the spring of 1913 an apparent negative heliotropic reaction by the germ-tubes was seen. The spores had been sown in a drop culture exposed to a unilateral illumination on a window sill and a high per cent. of the tubes had grown directly away from the light. Subsequent tests with controls in darkness substantiated the first observations. A search of the literature brought forth a single mention of a heliotropic reaction in germinating rust spores. Ward,[2] referring to a series of germination studies with urediniospores of *Puccinia dispersa*, has written: "My reasons for varying the direction of incidence of the light in certain cases were based on some results (as yet inconclusive) that the germ-tubes exhibit heliotropic curvatures."

That the sporidial germ-tubes of *Puccinia malvacearum* react negatively to daylight has been shown recently by Robinson,[3] but aeciospore germ-tubes of *Puccinia Poarum* were found to be indifferent. Germ-tubes of conidia of *Botrytis* sp. also grew away from light but those of other non-rust fungi tested *Alternaria* sp., *Penicillium glaucum* and *Peronospora parasitica*, were indifferent according to Robinson.

During the past fall, 1914, the study of the effect of light on germinating urediniospores of *Puccinia Rhamni* was again taken up. Urediniospores were obtained at first from the field and stored in gelatin capsules and later from cultures on oat plants grown in the greenhouse. The urediniospores of this species are especially suitable to daylight tests on account of their quick germination and the rapid growth of their germ-tubes. In the tests made the germ-tubes averaged in growth once to twice the spore length in as many hours and four to six times in three to four hours.

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[1] Read before the American Phytopathological Society at the Philadelphia meeting, January 1, 1915.

[2] Ward, H. M. Ann. Bot. **16**: 267. 1902.

[3] Robinson, W. Ann. Bot. **28**: 331–340. 1914.

The following method of germination was chiefly used. Dry urediniospores were dusted over the surface of a drop of a 5 per cent non-nutrient gelatin placed on a glass slide in a Petri dish containing a moist filter paper. The Petri dish was then placed in a dark box on a window ledge with an aperture, 2.5 cm. in diameter, towards the window. All tests were made with diffused daylight. Controls were maintained in darkness.

In all of the ten or more tests that were made with an exposure of four or five hours to a unilateral illumination more than four-fifths of the germ-tubes responded negatively to the light stimulus. A count of some 200 germ-tubes in three tests gave an average of 86 per cent that had grown away from the light (*fig.* 1*a*). A small part of the remainder had grown towards the light and a large part were recorded as transverse to the incidence of light. The germ-tubes of the controls in darkness grew in all directions (*fig.* 1*c*).

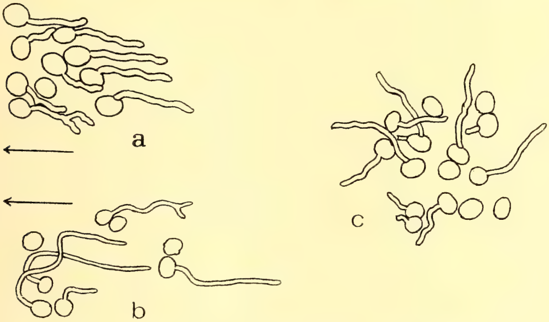

FIG. 1. Germinating urediniospores of *Puccinia Rhamni; a* and *b*, exposed for three hours to a unilateral illumination; *c*, germinated in darkness. The arrows indicate the direction from which light was admitted.

Urediniospores of *Puccinia Rhamni* have from six to eight germ-pores that are distributed at approximately equal distances apart over the spore surface. A study of the germ-tubes that had grown away from the light showed that the large majority of them has issued from pores located on the part of the spore wall farthest from the light (*fig.* 2). Others that had issued from pores on the part of the wall towards the light or transverse to it had soon changed their direction of growth by a turn of 90° or 180° and had then grown away from the light (*fig.* 1*b*). The incidence of light, therefore, not only had a pronounced effect in determining the direction of growth of the germ-tubes, but also determined to a considerable degree the approximate part of the spore wall at which the germ-tube issued, *i. e.*, the part farthest from the light. The other pores on the shaded part of the spores were likewise stimulated to some degree and a noticeable swelling of their gelatinous contents was often apparent. Sometimes two germ-tubes issued from the shaded side but one of them soon outgrew the other. When the spores were germinated in darkness the tube issued from any one of the pores.

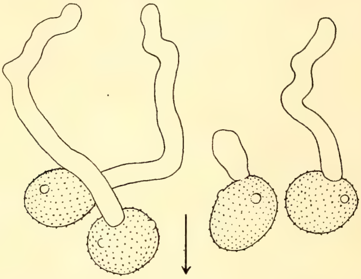

Fig. 2. Germinating urediniospores of *Puccinia Rhamni* after an exposure of three hours to a unilateral illumination. The arrow indicates the direction from which light was admitted.

No theory to account for the presence or significance of more than one germ-pore in urediniospores has been advanced. If a possible explanation in terms of advantage is permissible, it seems reasonable to assume that a germ-tube in nature arising from the shaded side of the spore, adjacent to the leaf surface, would be in a more advantageous position to effect an entrance into a stoma than one arising from the non-shaded side, away from the leaf. A urediniospore with several pores should, therefore, have a better chance of producing an infection than one with a single pore.

The importance of these light reactions of germinating urediniospores from a pathological viewpoint may be found in the possible explanation of certain phases of infection that they suggest. The nature of the stimulus, or combination of stimuli, that is responsible for the stomatal entrance of germ-tubes of rust spores and of other parasitic fungi has not been definitely ascertained. The more general belief is that the host exerts a positive chemotropic influence on the germ-tube, but the failure of attempts to prove that chemotropism plays an important part in this process has been practically universal. If a chemotropic attraction by the host is to be assumed in the rusts it must be considered a very general property of plants and not a specific property of the hosts alone, since urediniospore germ-tubes of a number of rusts have been shown by Gibson[4] to enter the stomata of non-hosts as well as those of their hosts. That positive hydrotropism may partially explain stomatal entrance is suggested by the work of Balls[5] and of Fulton[6] but it is doubtful that this can be a factor of primary importance. What part the action of light may play in bringing about the stomatal entrance of the urediniospore germ-tube is as yet a matter of conjecture. It seems quite probable, however, that a continued turning away from light may serve to bring the germ-tube into close contact with the surface of the host and be chiefly responsible for its entrance into the stomatal opening. This is, of course, only a preliminary stage of infection. The success or failure of the attack on the host tissue from the substomatal chamber cannot be directly influenced by light.

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[4] Gibson, C. M. New Phytol. **3**: 184–191. 1904.

[5] Balls, W. L. New Phytol. **4**: 18–19. 1905.

[5] Fulton, H. R. Bot. Gaz. **41**: 81–108. 1906.

A WOODY STEM IN MERREMIA GEMELLA INDUCED BY HIGH WARM WATER

Frank C. Gates

In the large swamp a little west of Los Baños, Laguna, Philippine Islands, *Merremia gemella* (Burm.) Hallier f. (Convolvulaceae) is a common vine which sometimes grows over *Phragmites vulgaris* (Lam.) Trin. and Sesbania (nearest *S. sesban* (L.) Merr.). As both of these species grow in water the greater part of the year and Merremia is neither a parasite, like Cuscuta, nor an epiphyte, its roots must also be under water. This is not a normal condition for Merremia but it does adapt itself and lives. Consequently, even near the center of the swamp, one finds festoons of Merremia draped over the Phragmites and Sesbania, even when they are growing in water a meter deep.

Usually the stem of *Merremia gemella* in dryland thickets and as a weed in cultivated land is herbaceous and dies down each year. Under the conditions obtaining in the swamp, the first and perhaps most striking thing seen is the prominent woody stem which may be as much as 20 mm. in diameter.

As a seedling Merremia must start in the ground and is continually dependent upon the ground for certain mineral salts. The seedling develops into a vine, rapidly making its way to the upper story of vegetation and spreading out over whatever happens to be there. To do this the normal herbaceous stem appears to be sufficient under ordinary conditions. Most of the swamp area is submerged during the rainy season, so that the roots of Merremia are under water. In ordinary years they are not generally far under water—less than 25 cm. rather than more. The sources of the water in the region are bubbling hot springs, whose temperature is usually between 70 and 90° C. The water soon becomes cooled to a temperature between 30 and 40° C., which several plants withstand.

The high water of 1914 was very high,—more than 1.5 meters over considerable area. The water covered the hot springs, which however continued to heat up the water in their vicinity. In the immediate vicinity of the hot springs the plants were killed outright and deleterious effects spread considerably further. Six weeks later, at the end of October, the temperature of the water a kilometer away from the hot springs was 27° C., whereas the surface water over them was 37 to 42° C.

All of the Merremia in the vicinity of the hot springs was killed. Further away where the temperature did not go much above 30° C. the effect was to stimulate the growth of a woody stem. The secondary thickening was very irregular, forming what is known as anomalous structure. Beneath the warm water level, there was only a very little secondary thickening present, of the same general type as that above the water but much poorer in amount and diversity of anomalous structure. The xylem vessels were noticeably larger in the part of the stem above the water. The bark above the water was rugged, but beneath the water it was quite smooth and compact. It was frequently covered with putrifying bacteria, but did not produce aerenchyma. When the stem was submerged during only the highest part of the high water and especially when it was twining around a stem of Sesbania, at the places where aerenchyma was produced by the Sesbania, short (less than 25 mm.), horizontal, clinging roots were developed from the stem of the Merremia. Entirely above the water

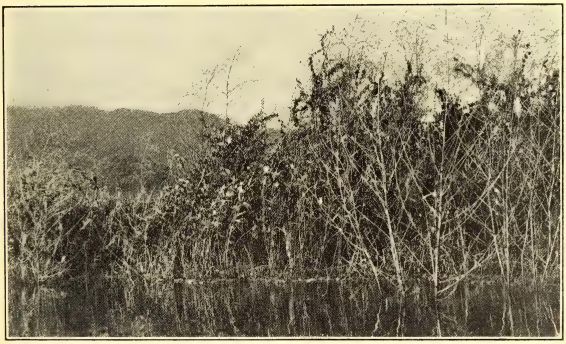

FIG. 1. The edge of the creek in the center of a swamp, showing *Merremia gemella* climbing on *Sesbania*. *Sesbania* in fruit is nearly leafless. Los Baños, P. I. October 31, 1914.

where no aerenchyma was produced by Sesbania, no clinging roots were present on the Merremia. These roots closely covered the aerenchymatous surface but did not seem to penetrate it.

Lack of sufficient air in the lower part of the stem and in the roots was obvious. The smaller size and number of the vessels in the lower part, the simpler structure as well as the smaller amount of starch present in the lower part of the stem showed that the conduction of the food downwards was also affected by the continued submergence of the stem in the warm water. The results were somewhat similar to girdling in that the phloem was very much congested in the upper part of the stem.

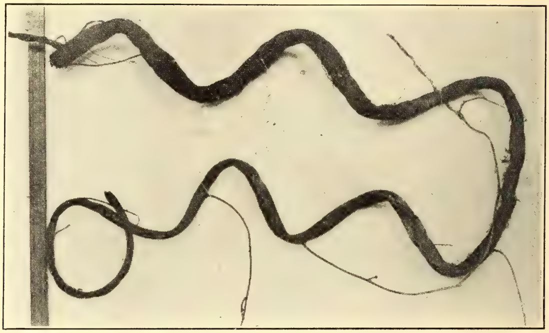

FIG. 2. A piece of the stem of *Merremia gemella* from near the center of the swamp. The upper and larger part was not submerged, while the lower and thinner part was covered with warm water. Los Baños, P. I. October 31, 1914.

If the roots developing from the stem had penetrated the supporting host, a first stage in the development of parasitism would have been apparent, but such was not the case.

Of a number of stems measured, the lowest part, which was beneath the water, varied from 4 to 10 mm. in diameter, while that above the water was from 10 to 20 mm. in diameter. The greatest difference found in a single plant was 6 mm. beneath and 18 mm. above the medium high water level.

LOS BAÑOS, PHILIPPINE ISLANDS

PICRO-NIGROSIN, A COMBINATION FIXATIVE AND STAIN FOR ALGAE[1]

OTIS F. CURTIS AND REGINALD H. COLLEY

Pfitzer (1) used a saturated aqueous solution of picric acid, to which he added a varying amount of an aqueous solution of nigrosin, to kill and stain minute forms, such as diatoms and small algae, under the cover glass. Freeborn (2), Pianese (3), and Johnston (4), and other zoological investigators have employed picro-nigrosin alone, or in combination with other stains, with excellent success, but there is little reference to its use by botanists. The writers, by independent study, have found it a valuable stain for filamentous algae and for younger tissues of forms with denser structure, and believe that the results of their experiments, which confirm Pfitzer's results in practically every detail, are of sufficient interest to warrant this brief note.

The combination fixing and staining solution, which may be used several times without apparent deterioration, was prepared by saturating a saturated aqueous solution of picric acid with Gruebler's water-soluble nigrosin. Material was placed in this solution for from three to twenty-four hours, rinsed in water to remove the excess stain, and run through the grade alcohols with half hour intervals, to glycerine, clove oil, Venetian turpentine or balsam. The general result, regardless of variations in the method after the material has been run through the alcohols, is a blue-black coloration of the nucleus and nucleolus, a faint blue in the pyrenoid and cytoplasm, an almost colorless chromatophore, and a colorless cell wall. The stain is unusually transparent, differentiating the nuclear structure in cells and tissues with dense cytoplasm. For immediate class use, algae were studied in 70 per cent alcohol or transferred to a mixture of alcohol and glycerine which served to clear fairly well and prevented the mount from drying up during the laboratory exercise. Some of the best results were obtained with material transferred from absolute alcohol to clove oil and examined in the latter. Excellent permanent mounts were made by following Chamberlain's Venetian turpentine method

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[1] Contribution from the Marine Biological Laboratory, Woods Hole, Massachusetts.

(5), or by running through the grades of xylol into a weak solution of balsam in xylol, which was then allowed to thicken gradually to the right consistency for mounting. The stain keeps well in both media. Shrinkage and distortion, especially in young cells, is practically negligible when reasonable care is used in handling material. For class use the Cyanophyceae, Nemalion, and forms without large vacuoles can be mounted directly in balsam from clove oil or xylol with little or no shrinkage. Forms with large vacuoles may show some distortion when transferred from absolute alcohol to clove oil or xylol but the amount of shrinkage may be decreased by making the change more gradually. Apparently there is no need of carefully timed treatment as there is rarely any danger of injury or overstain; for, with most material, the results vary little whether the time of fixing and staining be twelve hours or three days. A few forms, particularly some of the red algae, should rarely be left in the solution more than twelve hours as they soften very quickly; other forms, although they may seem to have been softened and spoiled are hardened and restored to normal appearance in the higher alcohols. Pfitzer suggests the use of ammonia to take out the excess stain, but it has been found sufficient in most cases to rinse in water or simply to lengthen the time in the lower alcohols.

Experiments were conducted to determine the value of the combination killing and staining solution on widely separated genera of the algae, including Oscillatoria, Lyngbya, Volvox, Spirogyra, Zygnema, Oedogonium, Ulva, Enteromorpha, Nitella, Ectocarpus, Sphacelaria, Fucus, Nemalion, Callithamnion, Ceramium and Polysiphonia, and on various Diatoms, several genera of the Agaricaceae, and young root tips. The stain proved very satisfactory for the algae, with the exception of Ceramium and Polysiphonia, and the Diatoms, but further work must be done with fungi and higher plant tissues before definite conclusions can be stated.

The value of the method may be indicated by a brief statement of the general results on some of the forms studied: In Oscillatoria and Lyngbya the chromatin granules are brought out distinctly because they stain more deeply than the rest of the cell contents. In Enteromorpha the nucleus is clearly differentiated; the pyrenoid is stained faintly and appears to stand in a colorless area, the chromatophore, which is hardly stained at all. In Sphacelaria the young propagulae show practically no shrinkage, even when material is removed from absolute alcohol to clove oil and then to balsam. The general nuclear phenomena of propagula-formation may be followed without sectioning. In unfertilized Fucus eggs the nucleus stands out sharply, surrounded by radiating cytoplasmic strands. In fertilized eggs, since the brown color is taken out, one can follow the enlargement of the nucleus and its elongation, the appearance of the centrosomes and the spindle figure, and the reorganization of the daughter nuclei. The actual fusion of the egg and sperm nucleus is difficult to observe. In the two weeks old embryo, the structure of the multicellular body resulting from the division of the fertilized egg shows well; in the rapidly growing rhizoidal tips, the spindle figures are nicely differentiated. In Nitella the nucleus and nucleolus in the ripe oosphere are clearly visible through the spiral corticating cells. The latter, as well as the young cells and growing tips, show practically no shrinkage. In all cases diatoms are cleared so that the nucleus and general structure may be studied with ease.

Further work is being carried on with fungi and with other material which indicates that the combination killing, fixing and staining solution may have a much wider range of usefulness. The experiments so far conducted apparently establish the following points of special advantage:—

There is an unusually wide range in the time of treatment,—three hours to three days; but, even in the long period, there is little danger of overstaining or overfixing.

Since excess stain and acid are removed in most cases by rinsing in water or in the process of running through the alcohols no special washing is required.

The stain is quite insoluble in strong alcohol; therefore material may be kept for a long time in the higher alcohols.

It is an exceptionally transparent stain which differentiates the nuclear structure in cells with dense cytoplasm.

The fixation is usually good and if sufficient care is taken in subsequent treatment excellent permanent mounts can be made.

The stain keeps well in Venetian turpentine or balsam.

These facts suggest that the method, which is unusually simple, may be valuable in preparing material for class work and in preliminary cytological work on material in toto, as well as convenient for demonstrating cell structures which would be completely obscured by such a stain as Heidenhain's iron alum hematoxylin.

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5. **Chamberlain, C. J.** Methods in Plant Histology. Ed. 2. 81–84. 1905.

NORMAL AND ABNORMAL PERMEABILITY

W. J. V. Osterhout

Some investigations by the writer on the permeability of protoplasm have recently been reviewed by Höber.[1] It seems evident that his views depend in part on a misunderstanding in regard to the relative amount of permeability under normal and abnormal conditions, and in part on a misunderstanding of the author's meaning.

The investigations of the writer show that the permeability of cells of Laminaria can be determined by measuring their electrical resistance. In sea water the normal permeability remains unaltered for a long time: in a solution of NaCl of the same conductivity as the sea water the permeability rapidly increases (as shown by the lessened resistance): this increase continues until the tissue is dead. The alkali salts behave in general like NaCl. The addition of a definite amount of $CaCl_2$ to the NaCl prevents (at least for some time) the increase of permeability which would occur in pure NaCl.

On this Höber comments as follows: "Osterhout schliesst aus diesen Versuchen, dass der lebende Protoplast von den Ionen der Alkalisalze leicht zu durchdringen ist. Aber ich halte diese Folgerung auch hier wieder für nicht richtig. Vielmehr zeigen die Versuche nur, dass die Laminariazellen ein Widerstandsmaximum aufweisen, wenn sie sich unter den physiologischten Bedingungen des Aufenthalts in Meerwasser befinden, und dass sie den Übergang in abnorme Bedingungen mit Widerstandssenkung, also mit Permeabilitätsverringerung[2] beantworten, vergleichbar der, die auch den Tod begleitet."

As has been suggested, Höber's views are due, in part at least, to a misunderstanding and it seems desirable to indicate briefly wherein this lies.

The writer has laid emphasis on the rapid penetration of pure NaCl and similar salts because this penetration was expressly denied by Overton and was used by him as one of the chief evidences for the lipoid theory of permeability. As is well known Overton reported that cells of Spirogyra when plasmolyzed in NaCl did not recover, thus showing that the salt did not penetrate. The writer repeated this experiment and found that penetration occurred: this conclusion was confirmed by measurements of electrical conductivity. The writer laid emphasis on these facts in order to call attention to the misleading character of Overton's conclusions and to indicate that the lipoid theory does not rest on a firm foundation.

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[1] Die physikalische Chemie der Zelle und der Gewebe. 4te Aufl. 1914, S. 362 ff.

[2] "Permeabilitätssteigerung" is evidently meant.

The writer, however, has nowhere stated, as Höber seems to think, that the penetration in pure NaCl represents the normal state of things. He has, on the contrary, emphasized the fact that in sea water (and in other solutions in which life can be maintained for a long time) the permeability is much less than in pure NaCl because $CaCl_2$ (and other salts) are present to antagonize the action of NaCl.

On the other hand the permeability in sea water (which the writer regards as the normal permeability of Laminaria), while less than that in NaCl, is by no means the minimum permeability, nor is the resistance in sea water the maximum resistance, as Höber seems to suppose. The net resistance of a cylinder of tissue, which in sea water is 800 ohms, may rise to over 1,600 ohms in a solution of $La_2 (NO_3)_6$ which has the same conductivity as sea water. In other words the maximum resistance is at least twice as great, and the minimum permeability at least 50 per cent less than that found in sea water.

Under these circumstances we are not justified in speaking (as Höber does) of the "normal impermeability" of protoplasm to salts, unless it is understood that we mean by "impermeability" merely a rate of penetration considerably lower than that found in dead cells. In the present instance it was found that the resistance of the cylinder of dead tissue was about 100 ohms, which was approximately the same as that of a cylinder of sea water of the same size. We can not, however, conclude that the resistance of the living protoplasm (in sea water) is just eight times as great as that of dead cells, for in the living tissue a part of the current passes between the protoplasts, travelling in the intercellular substance in which protoplasmic masses are imbedded.

It is evident that much confusion will disappear when such terms as permeability and impermeability can be quantitatively defined in all cases. The writer has sought to formulate quantitative expressions,[3] not only for permeability but also for such conceptions as injury and vitality. It is to be hoped that the study of permeability may before long be placed on a quantitative basis.

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[3] Cf. Science, N. S. **40**: 488. 1914.

A SIMPLIFIED PRECISION AUXANOMETER

W. T. Bovie

In a former paper[1] the writer described a precision auxanometer capable of registering very small increments of growth. The improved auxanometer here described works on the same principle as the previously described instrument. It consists essentially of a device which is carried upwards as the plant grows. When the device has moved a certain distance, it closes an electric circuit, which operates the recording pen of a chronograph. As pointed out in the paper referred to above, this arrangement makes it possible to have the recording mechanism at any desired distance from the plant and also to make simultaneous records of a number of plants or of various parts of the same plant.

The design of the instrument has been changed in order to make it suitable for the class room. The new auxanometer is light, compact, easily and quickly attached to the plant, and, as the force of gravity is not utilized for moving any of its parts, it may be set at any angle with the vertical, so that growth in any direction may be measured and recorded. A slight change in the electrical mechanism has made the instrument more reliable. The experimental model ran the entire summer in the garden exposed to the weather, the brass case being open part of the time so that the internal mechanism was exposed.

A rack and pinion has been substituted for the micrometer screw and nut of the instrument described in 1912. This change has been made in order to lower the cost of manufacture. For special research work, where records of very small variations in the rate of growth are to be made, the micrometer screw model should be used; but it has been found that for class work, and for research work where great precision is unnecessary, the rack and pinion gives satisfactory results. In the instrument with the micrometer screw the escapement wheel is fastened to the top of the micrometer screw and consequently the control of the motion of the micrometer screw is positive. As will be seen presently, the instrument described here does not possess this valuable feature. The difficulty has been met by making the instrument less sensitive. The improved instrument records each 0.1 mm. of growth, and the errors due to the loss of motion of the various parts of the mechanism are within the range of other unavoidable errors. Making the instrument less sensitive has an advantage for classroom work. It has been found that if the instrument is more sensitive the task of counting the checks on the chronometer record and plotting the curves requires too much of the student's time. The record of the

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[1] Bot. Gaz. **53**: 504–509. 1912.

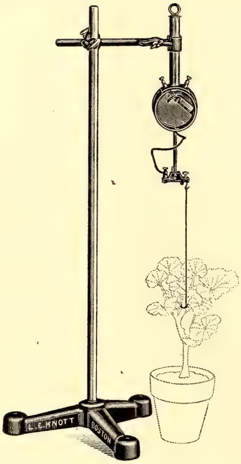

FIG. 1. A simplified precision auxanometer.

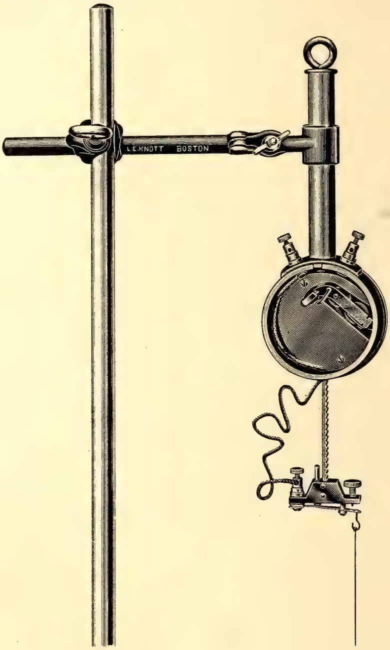

FIG. 2. Precision auxanometer, showing external details.

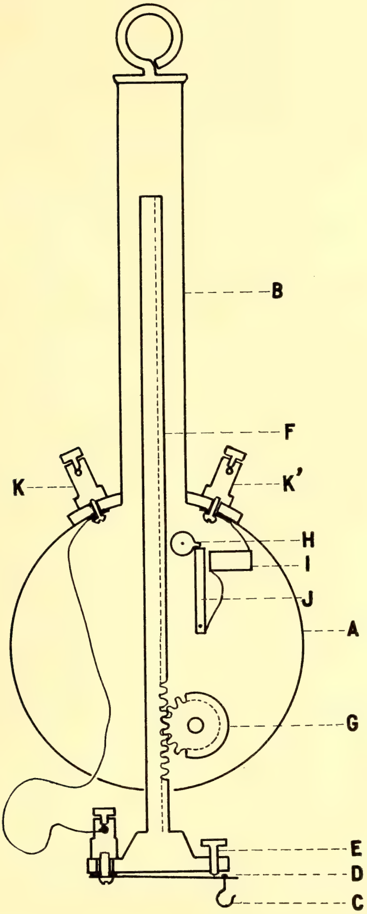
B
F
K
K'
H
I
J
A
G
E
D
C

FIG. 3. Precision auxanometer, showing construction (see text for explanation).

improved instrument, because of the smaller number of checks, requires less time for plotting the curves.[2]

The principle of the new instrument will be understood from the accompanying figures. Figures 1 and 2 show the exterior while figure 3 shows the construction. *A* and *B*, fig. 3, is the auxanometer case. The plant is attached by a fine wire to the hook *C*, which is fastened to a flat spring. A slight tension on the wire bends this spring downwards. As the plant grows the spring moves upwards and touches the screw *E*, thus closing the electrical circuit at *D*, which up to this time has been open, since the other end of the spring and its binding post are insulated from the block supporting *E*. The tension on the spring, which is controlled by turning the screw *E*, is so slight as not to affect the rate of growth.

Through the terminals *K* and *K′* the instrument is connected to an battery and chronograph. When the contact is made at *D*, current flows through the instrument and the chronograph and the pen of the chronograph makes a record. The current through the instrument flows from the terminal *K* to the binding post of the flat spring, along the flat spring and on to the screw *E*. From *E* it flows through the rack *F* to the pinion wheel *G*, through a train of gear wheels and their supporting framework (not shown in the diagram) to the escapement wheel *H*, then on to the armature *J* and through the electromagnet *I* to the terminal *K′*. The electromagnet *I* is thus energized, and attracts the armature *J*, which is held against *H* by a spring. When the armature *J* moves over against the electromagnet it releases the pin on the escapement wheel, and at the same time opens the circuit, since the contact between *H* and *J* is broken. The electromagnet is thus deenergized, the armature *J* returning to its position against the escapement wheel *H*. The escapement wheel is connected with the pinion wheel *G* through a train of gear wheels and the pinion wheel is under the tension of a clock spring. Therefore, when the pin on the escapement wheel has been released by the movement of the armature

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[2] The L. E. Knott Apparatus Company of Boston have developed a special chronometer which in addition to making the records, automatically draws a curve of the rate of growth. The writer has had no part in the development of this chronometer other than to test the experimental model. The instrument does its work well, but it has the disadvantage that the record of only one auxanometer can be made at one time. With the chronometer which the writer has devised for his own use (a drum rotated by an eight-day clock) the record of six auxanometers can be made at one time.

J, the escapement wheel turns. At the end of one revolution the pin of the escapement wheel is again engaged by *J*. For each revolution of the escapement wheel the pinion wheel *G* turns sufficiently to lift the rack *F* 0.1 mm. This upward movement of the rack causes the contact at *D* to open and the plant must grow 0.1 mm. before it will close again. Thus it will be seen that a record is made for each 0.1 mm. of growth, and from the chronograph records the rate of growth may be determined.

It will be noticed that the circuit is closed at *D* but never broken there. The break is between *H* and *J*. This avoids arcing and the pitting of the terminals at *D*. It prevents sticking and actual falsification of the record due to changes in the length of *E*. Making the break between *H* and *J* has another advantage: in setting up the instrument it is only necessary to attach the plant at *C*, and make the electrical connections. The escapement wheel is released and continues to revolve until the rack *F* has lifted sufficiently to open *D*. The instrument then begins to record growth, no further adjustment being necessary.

As the rack *F* moves up into the case the clock spring which actuates *G* unwinds. It is rewound by grasping the lower end of *F* and pulling it down. As the rack is 15 cm. long, 15 cm. of growth may be recorded at one winding.

The expense of the experimental work on this machine has been borne by the L. E. Knott Apparatus Company of Boston and their mechanician, Mr. Browne, is to be credited with the development of the design so as to minimize the cost of manufacture.

Harvard University,
Laboratory of Plant Physiology

THE MUTATIONS OF OENOTHERA STENOMERES

H. H. Bartlett

This preliminary article on the mutations of *Oenothera stenomeres* Bartlett is presented now in order to substantiate certain statements in a paper contributed by the writer to the symposium on the genetic relationships of organisms, held at the recent Philadelphia meeting of the Botanical Society of America. The papers of the symposium will appear in this Journal next month. Some of the mutations of *Oe. pratincola* Bartlett, which were also referred to, are dealt with in an article which have been published elsewhere.[1] A brief statement in regard to the mutability of *Oe. stenomeres* may be found in an earlier number of this journal[2] to which reference must be made for a full characterization of the species. The two mutations are mut. *lasiopetala* and mut. *gigas;* the former has occurred five times in the cultures of the first four generations, the latter but once. These cultures may be summarized as follows:

P			Unknown.
F_1	1910		A single plant, culture No. 32, transplanted into the garden from the wild and self-pollinated; f. *typica.*
F_2	1911	∞	Progeny of No. 32, 10 plants; f. *typica.*
F_3	1912	∞	Progeny of No. 32–4, 8 plants; f. *typica.*
F_3	1914		Progeny of No. 32–4, 4 plants; 3 mut. *lasiopetala,* 1 f. *typica.*
F_3	1912	∞	Progeny of No. 32–6, 8 plants; f. *typica.*
F_3	1914		Progeny of No. 32–6, 80 plants; f. *typica.*
F_3	1914		Progeny of No. 32–9, 7 plants; 1 dwarf, 6 f. *typica.*
F_4	1913		Progeny of No. 32–4–6, 106 plants; 1 self-sterile, 1 mut. *lasiopetala,* 1 mut. *gigas,* 103 f. *typica.*
F_4	1914		Progeny of No. 32–4–6, 96 plants; 1 mut. *lasiopetala,* 95 f. *typica.*

The object of growing the cultures of 1910, 1911 and 1912 was primarily systematic, and the garden space was small. Consequently only a few plants were brought to maturity. In the summary above I have indicated by the sign ∞ the cultures in which germination was normal and from which most of the seedlings were discarded. In the other cultures germination was poor, and all the plants were kept. It will be noticed that mutations were found only in the cultures characterized by poor germination. The writer usually sows the seeds from two or three capsules (400–900 seeds) in each germination pan. Unfortunately, the seeds were not counted for any sowings of *Oe. stenomeres*. In 1913, the first year in which plenty of garden space was available, all of the seedlings obtained were kept until maturity, and in that year the two mutations, mut. *lasiopetala* and mut. *gigas*, were discovered. In the following year several packets of old seeds, which has been left over from previous plantings, were sown, with the result that one lot of possibly a thousand old seeds gave only four plants, of which 3 were mut. *lasiopetala* and one was forma *typica*. The same seeds, two years before, had germinated normally and the young seedlings appeared uniform. Eight of them, carried to maturity, were all f. *typica*. It is clear that mut. *lasiopetala* is a mutation of infrequent occurrence, but that the seeds which give rise to it are for some reason longer-lived than those of f. *typica*. The phenomenon of selective mortality is probably very important in the operation of natural selection. In the article on *Oe. pratincola* mut. *nummularia* (l. c.) very clear evidence of differential fermination and selective mortality is presented. In the case of *Oe. stenomeres* mut. *lasiopetala* the latter phenomenon seems to account for the unexpected composition of small cultures.

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[1] Bartlett, H. H. Additional Evidence of Mutation in Oenothera. Bot. Gaz. **59**: 81–123. 1915.

[2] Bartlett, H. H. An Account of the Cruciate-flowered Oenotheras of the Subgenus Onagra. Amer. Journ. Bot. **1**: 226–243. 1914.

Oe. stenomeres mut. **lasiopetala** nom. nov.[3]

Before the rosettes of this mutation have attained a diameter of 2 cm. they may be distinguished from the type form by their more densely pubescent, broader, sometimes almost orbicular leaves. The leaves of f. *typica* are ovate. Differences become more pronounced as the plants grow older. The mutation remains persistently in the rosette condition. The rosettes of the type, on the contrary, are transitory, for the species is an annual. The mature foliage of the mutation is so densely pubescent that it looks gray in comparison with the green foliage of the type.

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[3] The name of this mutation was originally published as *Oe. stenopetala* mut. *lasiopetala* (Amer. Journn. Bot. 1: 237. 1914). The specific name *Oe. stenopetala* had been used in the writer's paper as originally submitted for publication, but was later altered to *Oe. stenomeres* on account of the prior publication of *Oe. stenopetala* Bicknell. In one place the preempted name escaped correction in the proof. The oversight is especially regretted because Mr. Bicknell's new species is likewise one of the cruciate Onagrae. It is not, however, at all closely allied to *Oe. stenomeres*, not has it yet been made the subject of experimental study.

Among the five primary mutations and 44 F_1 plants of mut. *lasiopetala* which have been under observation only one plant has so far flowered. All of the few plants which became cauline formed rosettes at the ends of the stem and branches instead of flowering. Only in the case of the plant shown in figure 1, a single branch flowered late in the fall of 1913 and produced three capsules of seeds. All of the other branches, and the main stem, bore rosettes in every way like the radical rosette. In 1914 no seeds were obtained. The most striking feature of mut. *lasiopetala*, the hairiness of the petals, has therefore been observed in only one plant. The pollen was examined and was found to have three germination points, as in the parent form. Perhaps one grain in a hundred was found to have four points, as in mut. *gigas*. In f. *typica* 60 to 80 per cent of the pollen grains are perfect; in mut.

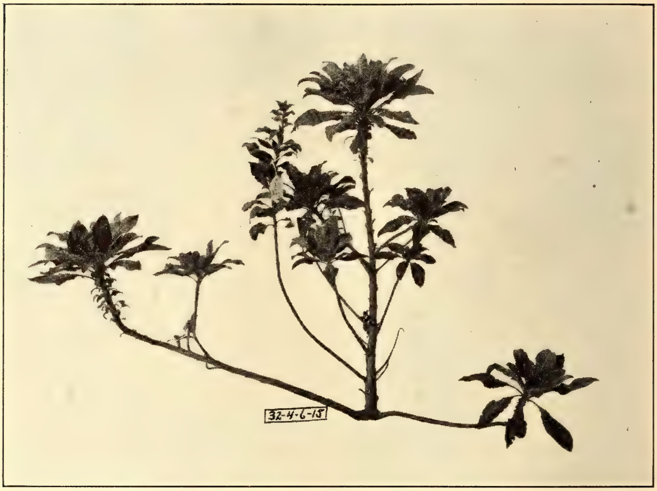

FIG. 1. The original plant of *Oe. stenomeres* mut. *lasiopetala*, no. 32-4-6-15. The label on the ground is 10 cm. long.

lasiopetala between 40 and 50 per cent are perfect, the remainder shrivelled and defective.

The F_1 progeny of mut. *lasiopetala*, from self-pollinated seeds of no. 32–4–6–15, may be summarized as follows:

	Seeds	Plants	F. *typica*	Mut. *lasiopetala*	Other muts.
Capsule *A*	78	26	—	—	—
Capsule *B*	122	39	—	—	—
Capsule *C*	156	51	—	—	—
Total F_1	356	116	70	44	2

The progeny consisted of 60 per cent. f. *typica* and 40 per cent. mut. *lasiopetala*. The two types were distinguished at a very early age. The mode of inheritance is comparable to that of two of the mutations from *Oe. Lamarckiana* Hort. namely *Oe. lata* de Vries and *Oe. scintillans* de Vries. The former is generally self-sterile for the reason that it produces no good pollen: de Vries,[4] however, succeeded in deriving a fertile strain from the cross *Oe. lata*×*semilata*, and found that its progeny by self-pollination consisted of *Oe. lata* and *Oe. Lamarckiana*, just as would have been the case if the pollen had been derived from *Oe. Lamarckiana*. In other words, it appears that the good pollen grains of *Oe. lata* are genetically the same as those of *Oe. Lamarckiana*, and do not carry the *lata*-characters. This conclusion is borne out by the fact that *lata*-like fertile hybrids between *Oe. lata* de Vries and *Oe. Hookeri* T. & G. cannot transmit the *lata*-characters through the pollen, and transmit to only a portion of their progeny through the eggs. *Oe. scintillans* acts like *Oe. lata* in every way; its pollen, moreover, is always sufficient for self-pollination.

Thus it appears that there is a class of mutations of which the eggs are of two kinds; one kind carries the characters of the parent species, the other kind the characters of the mutation. The pollen grains, however, appear to be of one kind only, and to carry the characters of the parent species. Is it not possible that the male gametes which carry the characters of the mutation are eliminated because of some physiological defect? If so, *Oe. lata* might be supposed to originate by the union of a *lata*-egg (itself constituting the true mutation) with a *Lamarckiana*-sperm. Then *Oe. lata* would be a heterozygote, and would produce gametes of two kinds. If the male *lata*-gametes were eliminated, the progeny of *Oe. lata* would consist of both *Oe. lata* and

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[4] Gruppenweise Artbildung, p. 256.

Oe. Lamarckiana, quite regardless of whether the pollen was derived from the heterozygous mutation or the homozygous parent species. A partial elimination of *lata*-eggs would explain the deviation from the expected 1 : 1 ratio, for the mutational form rarely constitutes a full half of the progeny. The pollen of *Oe. lata* is so much more highly defective than the ovules that this hypothesis seems not an improbable one. Gates and Thomas[5] have shown that in *Oe. lata* the cytological facts are in accord with such an explanation. The somatic number of chromosomes is 15, whereas in *Oe. Lamarckiana* it is but 14. *Oe. lata* therefore produces two classes of gametes, with 8 and 7 chromosomes, respectively. If two 7-gametes fuse, we have *Oe. Lamarckiana;* if a 7-gamete (presumably male) fuses with an 8-gamete (presumably always female) we have *Oe. lata.* Plants with 16 chromosomes have not been found. From this fact, and the data in regard to inheritance, we must assume that the male 8-gametes are eliminated.

There is clearly no reason why mutations which follow the *lata*-type of inheritance should not be viewed as progressive in their nature, even though they exist only as heterozygotes. Thus far only three such are known, *Oe. lata*, *Oe. scintillans* and the new *Oe. stenomeres* mut. *lasiopetala.*

Oe. stenomeres mut. **gigas,** mut. nov.

This rare mutation was found in the same progeny which included the first example of mut. *lasiopetala.* So far only one specimen has appeared. In the former paper (l. c., p. 236) it was merely referred to as a mutation with "very large, thick buds and short, thick fruits." It was even then thought to be a *gigas*-mutation, but was not named because a chromosome count had not yet been made. Mr. E. G. Arzberger has now found the chromosome number to be 28. Cytologically and morphologically there is perfect analogy between *Oe. stenomeres* mut. *gigas* Bartlett and *Oe. gigas* de Vries. Each is more persistently biennial than its parent form and has thicker, broader leaves, stouter stems, larger buds, thicker fruits, 4-pointed instead of 3-pointed pollen, and twice as many chromosomes. Each is characterized by a strongly rosuliferous rather than freely branched main stem, and bears its branches mainly in the axils of the rosette leaves. Each is marked by a greater range of variation than its parent form, in that it gives rise

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[5] Gates, R. R. and Thomas, Nesta. A Cytological Study of *Oenothera* mut. *lata* and *Oe.* mut. *semilata* in Relation to Mutation. Quart. Journ. Micr. Sci. **59**: 523–571. 1914.

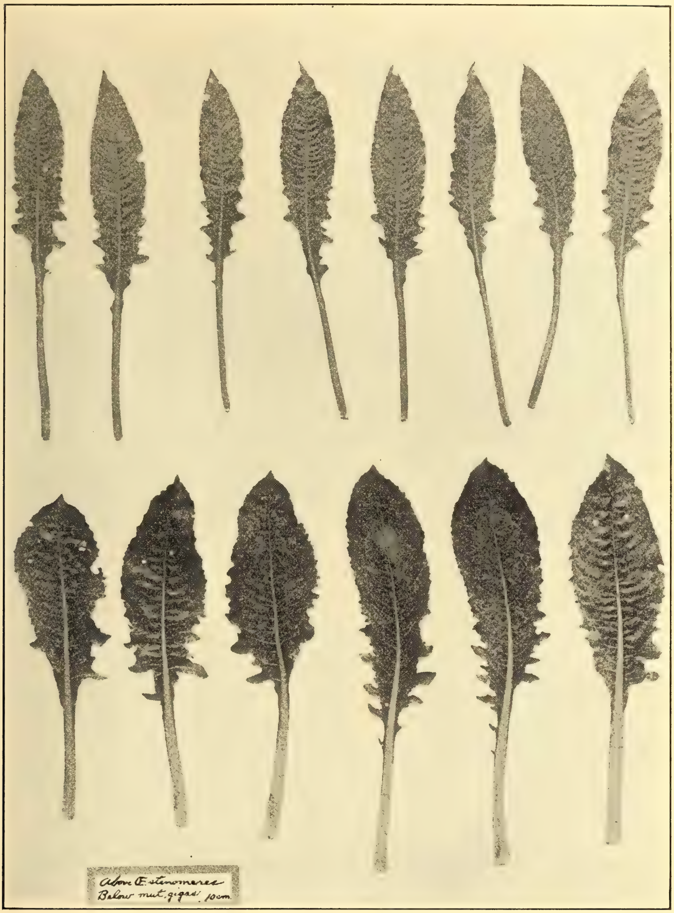

Above Œ. stenomeres
Below mut. gigas. 10cm.

FIG. 2. Rosette leaves of *Oe. stenomeres* f. *typica* (above) and mut. *gigas* (below). The label is 10 cm. long.

to narrow-leaved derivatives which otherwise have the *gigas*-characteristics.

From the very first the young rosettes of mut. *gigas* are larger and stronger than those of typical *Oe. stenomeres.* The leaves are half again as broad and much more densely pubescent. In nature the mutation would doubtless be a biennial, for the rosettes persist through early summer and have a tendency not to flower until late fall. It has already been remarked that f. *typica* is an annual. Among many hundred F_5 plants in the garden of 1914 it was difficult to find one from which rosette leaves comparable with those of the mutation could be obtained for the comparison shown in figure 2.

From figure 2 it will be seen that the contrast in leaf-breadth between parent and mutation is very great. Whereas the length of the longest leaves of either is about 300 mm., the breadth is about 40–45 mm. in f. *typica* and 70–75 mm. in mut. *gigas.* The same relative leaf-breadth obtains in the stem leaves, of which a typical series from each form is shown in figure 3. The buds of the mutation are a third again as long as those of f. *typica,* and more than proportionately thick (v. fig. 4). In extreme cases the buds, including the ovary, are 90 mm. long. The pollen grains have four germination points instead of three, and are therefore quadrangular instead of triangular. They are larger than the triangular grains of the type. A very few of them are triangular, as in the type, and a considerably larger number have five points. The pollen character was verified in the original mutation and in every plant of the F_1 progeny which bloomed. Gates has shown that the same pollen differences exist in the case of *Oe. Lamarckiana* and *Oe. gigas.*

The original plant of mut. *gigas* bloomed late in the summer, and few seeds were obtained. The F_1 progeny which they produced may be summarized as follows:

	Seeds	Plants
Capsule *A*	91	46
Capsule *B*	69	25
3 small capsules	85	31
Total F_1	245	102

Of the 102 plants only 63 reached maturity. The rest died or failed to flower. These 63 plants included 54 of typical broad-leaved mut. *gigas,* 6 narrow-leaved variations otherwise similar to mut. *gigas,* and 3 secondary mutations. Of the secondary mutations two were

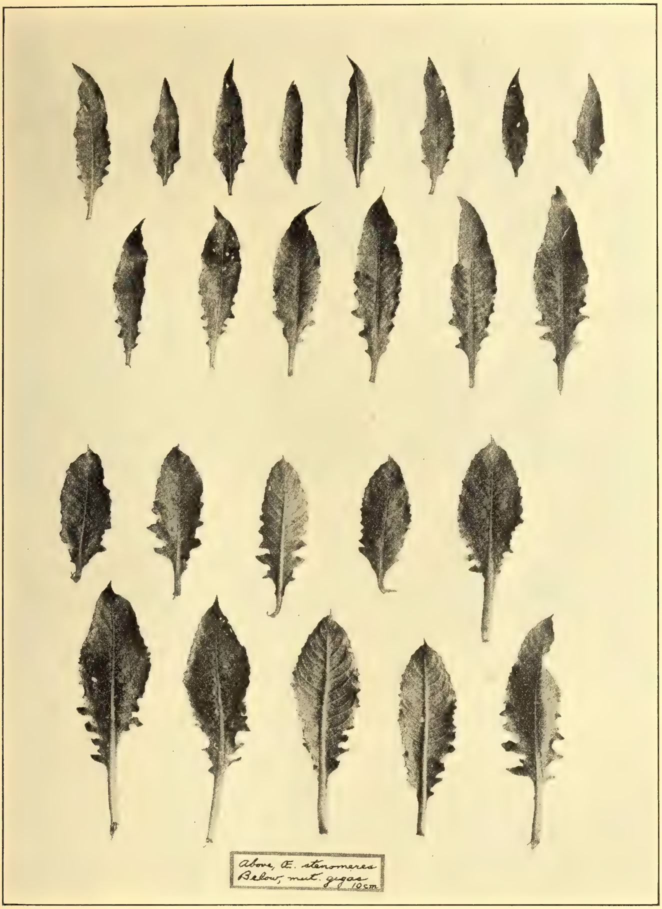

Above, Œ. stenomeres
Below, mut. gigas
10cm

FIG. 3. Stem leaves of *Oe. stenomeres* f. *typica* (above) and mut. *gigas* (below). The label is 10 cm. long.

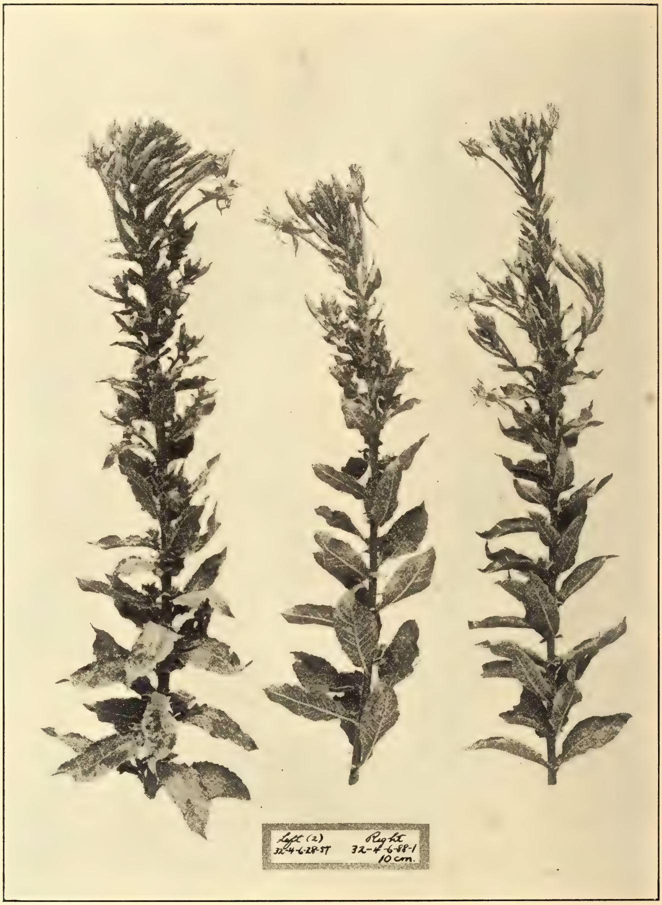

Left (2)
32-4-6-28-5T
Right
32-4-6-88-1
10 cm.

FIG. 4. Inflorescences of main stem of *Oe. stenomeres* f. *typica* (right) and mut. *gigas* (left). Inflorescence of basal branch of mut. *gigas* (center). The label is 10 cm. long.

dwarfs, and one corresponded to mut. *lasiopetala* from f. *typica*. It may be called *Oe. stenomeres* mut. *gigas*$_1$ *lanosa*$_2$. Not only were the petals hairy, as in mut. *lasiopetala*, but the filaments of the stamens alternate with the petals were densely lanose. The filaments of the other four stamens were glabrous. This plant was just coming into flower at the time of the first killing frost. It had to be removed to the greenhouse and does not give much promise of yielding seeds.

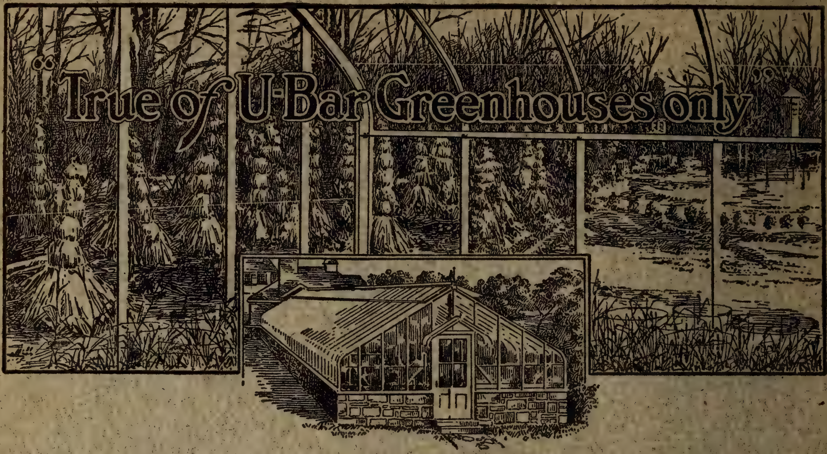
The plants which failed to flower appeared to have the *gigas*-characteristics. It will be remembered that *Oe. gigas* de Vries also bred true from the first appearance of the mutation.

CONCLUSION

The point which should be especially emphasized in connection with the mutations of *Oe. stenomeres* is that neither of them can be interpreted as the result of segregation following hybridization. *Oe. stenomeres* is an isolated cruciate species, related to none of the other cruciate species, and geographically remote from all of them. The peculiarities which characterize mut. *lasiopetala* are not known in any other species and cannot, therefore, be construed as having been introduced into *Oe. stenomeres* by hybridization with any other type. It seems more likely that *Oe. stenomeres* itself originated by mutation from some broad-petaled elementary species allied to *Oe. gauroides* Hornem., and that mut. *lasiopetala* constitutes a second mutation in the same direction. The occurrence of mut. *gigas* cannot be viewed as even remotely due to hybridization, for the characters of this mutation can be explained on a cytological basis.

Like most of the other *Onagrae*, *Oe. stenomeres* shows a considerable degree of pollen sterility. This sterility we may view as a sign of the prevalence of mutation in the group. Both mutation and hybridization bring about pollen sterility, and both processes are widely prevalent in the subgenus Onagra. It is illogical and unjustifiable to state, as certain authors have done, that the mutability is the result of the hybridity. It seems far more probable that the truth is exactly the opposite. Far from having proved that hybridization "explains" mutation, no one has yet even shown how hybridization *could* explain it. Finally, attention may again be called to the fact that *Oe. stenomeres* is a close-pollinated and practically a cleistogamous species in nature, and that its mutability was not discovered until the fourth generation of a guarded line was grown.

BUREAU OF PLANT INDUSTRY, WASHINGTON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MORPHOLOGY AS A FACTOR IN DETERMINING RELATIONSHIPS[1]

J. M. GREENMAN

The remarkable advance in our knowledge of relationship of organisms during the past quarter of a century is due primarily to extended studies in morphology in one phase or another. In this field data have been gathered from various sources, and there is at present a vast array of recorded observations many of which have been interpreted in terms of relationship and phylogeny. In fact among morphologists and taxonomists relationship and phylogeny have been leading questions of the day. So important is morphology in these matters that it may seem almost axiomatic to speak of morphology as a factor in determining relationships. Nevertheless, the methods of attack have been from such different standpoints and the significance of the results is such that I trust a general statement at this time may not be entirely out of place.

Naturally, I must speak mainly from the standpoint of a taxonomist, and furthermore I shall confine my remarks to a consideration of the higher plants. The general situation as to the morphology of the higher plants, both the gymnosperms and angiosperms, has been so carefully summarized and so admirably presented by Coulter and Chamberlain that no further words are needed to emphasize the importance of modern morphology to a sound taxonomy.

Notwithstanding the amazing progress in modern morphology and the fact that our knowledge of the life-history of individual organisms

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[1] Invitation paper read at the symposium on "The Genetic Relationship of Organisms" before the Botanical Society of America at Philadelphia, December 30, 1914.

[The **Journal** for February (**2**: 71–110) was issued 3 Ap 1915.]

and genetic relationship of groups of organisms has been materially broadened there has been thus far at least little application of the data obtained to affect, except to amplify, our present system of classification. The basis of classification of the higher plants today is still the old morphology, namely that of Caesalpini, John Ray, Linnaeus, Wolff, Goethe, De Candolle, Eichler, Gray, Bentham and Hooker, and Engler and Prantl; and it is expressed in epitomized form in Engler's *Syllabus der Pflanzenfamilien* (1912), and, so far as the angiosperms are concerned, in a somewhat ampliated form by Wernham in recent numbers of *The New Phytologist.* Our present system of classification has resulted from a very gradual development of the science, and along with it there has also developed an elaborate terminology which is more or less arbitrary and to a certain extent fixed. Furthermore, it expresses, as has often been said, the best interpretation of correlated knowledge of relationships up to the time of its publication.

Since the publication of Engler and Prantl's *Die Natürlichen Pflanzenfamilien* morphological research has been directed to various groups of plants a more detailed study of which might throw light upon their genetic relationship. Nowhere has this been more striking than in the Gymnosperms and nowhere I think may it safely be said to have been more effective from a taxonomic standpoint than in this group.

Investigations in plant anatomy have shown conclusively the importance of morphological anatomy in determining genetic relationship or phylogeny. Modern methods, through the work of Scott, Oliver, Arber, Jeffrey, Bailey, Eames, Sinnott and others, have made it possible and profitable to carry on researches along these lines. Morphological considerations of structural elements hitherto unused, or at least used to a very slight extent, have been demonstrated to be of value in determining relationship. How far such characters can be made use of in a general system of classification must remain until a correlation of all facts is effected and possibly for a future generation to decide. Personally, I am inclined to believe that these less obvious features must remain subsidiary to the more evident characters which are relatively easy of access.

There has also been a revival in the study of botanical embryology in its broader aspect, which just at present seems likely to be of the utmost importance in determining finally the relation of monocotyledonous to dicotyledonous plants. In fact morphological evidence is rapidly accumulating to show that the former group has been derived from the latter. Several writers have expressed this belief, but they have reached their conclusions from rather different points of view. I refer particularly to the recent contributions by Henslow and by Ethel Sargent. Miss Sargent's paper entitled "A Theory of the Origin of Monocotyledons, founded on the Structure of their Seedlings" points out that "the cotyledon of *Anemarrhena*, a liliaceous genus, contains two massive bundles which together form a tetrarch stele in the primary root"; and she interpreted this as probably due to "a fusion of two seed-leaves in some remote ancestor to form the single cotyledon of *Anemarrhena*." Seedlings of other Liliaceous genera were also examined, but this one she regards as representing a rather primitive type. Miss Sargent contrasts this condition with the condition shown by certain dicotyledonous plants, *e. g.*, species of *Anemone*, *Delphinium*, *Ranunculus*, *Trollius*, *Eranthis*, etc., "possessing seed leaves which are partially united, sometimes by one margin only, but more often by both." The conclusion therefore being that the monocotyledons have been derived from the dicotyledons through a fusion of the two cotyledons into one.

In this connection I may call attention to a very suggestive paper by Coulter and Land in a recent number of *The Botanical Gazette;* they record the results of a morphological study of seedlings of *Agapanthus umbellatus*, a South American amaryllidaceous plant, seedlings of which were found to possess more or less indifferently one or two cotyledons. This study has been continued, and a paper by the same authors was read by Professor Coulter at the recent celebration of the 25th Anniversary of the Missouri Botanical Garden. Although this second paper is not yet published Professor Coulter stated, "The general conclusion is that monocotyledony is simply one expression of a process common to all cotyledony, gradually derived from dicotyledony, and involving no abrupt transfer of a lateral structure to a terminal origin."

In other words morphological evidence has been presented by Miss Sargent that monocotyledony has resulted by a fusion of two cotyledons and according to Coulter and Land by the reduction and disappearance of one cotyledon. In either case the phenomenon is interpreted as indicating the origin of the monocotyledons from the dicotyledons.

While recent studies have been concerned mainly in determining the genetic relationship of the larger groups, yet, at the same time, attention has been given to the life history of individual species and groups of species, and to a discussion of the relationship and phylogeny of certain orders, such for example as the *Salicales*, a group recently investigated from an anatomical standpoint by Ruth Holden. This author takes objection to the relatively low position assigned the *Salicales* in the Engler and Prantl system; and she presents anatomical evidence, particularly concerning the primitive condition of the wood —namely vasicentric parenchyma and multiseriate rays—to show that this simple structure is due to reduction. It is interesting to note that in this case at least the anatomical evidence in stem-structure is supplemented by reduction in floral structure and also by entomophilous pollenation and porogamy.

Furthermore, it has been maintained, through comparative morphological studies, that the *Fagaceae* have been derived from ancestors allied to epigynous types of *Rosales;* and it is not unlikely, moreover, that a similar genetic relationship may be the case among the *Juglandales*. Several families of plants, for instance the *Araceae* in all probability represent lines of reduction and in this case possibly from some ancestral liliaceous type. It is not inconceivable also that the *Gramineae* may have developed from an ancestral stock allied to the present bamboo tribe. Among the dicotyledonous plants the Euphorbiaceae is generally regarded as a polymorphous family in which the floral structure in several genera has been very much reduced, as for example in *Acalypha* and *Euphorbia.*

In such groups as these a more thorough knowledge of the embryology and seedling anatomy would undoubtedly throw light upon genetic relationship as well as generic affinity. I think the objection taken to the relatively low position assigned to several groups in the Engler and Prantl sequence is well founded, but we must wait for additional morphological evidence and its proper correlation before satisfactory change in their position can be made.

Important as these embryological and anatomical studies are in determining genetic relationships, especially of the greater alliances, yet when it comes to a consideration of the relationship of different families and to generic affinity, taxonomy can be materially advanced by a more thorough knowledge of the floras of the different parts of the world. We need also to know more of the life-history of plants already recorded and to have our floras more fully represented in herbaria and garden. It is my belief that this can be done best through a co-operation of the larger botanical institutions. It is manifestly impossible for any one institution to cover the whole field with any degree of satisfaction, but it is possible for each institution either to direct its energies to a particular territory or to a particular group of plants. Thus it would be possible to place in the hands of the research student an abundance of the necessary kind of material for comparative morphological study and afford the opportunity to determine the range of variability.

Our conception of the origin and limitation of species has been so modified by recent investigations in plant breeding, mutation, hybridization, etc., that relationship in a line of evolutionary sequence is an extremely complex problem. Nevertheless the new morphology, the intensive study of cell-phenomena, and experimental work have done much to advance our knowledge of the relationship of organisms; and it is not improbable that within a comparatively short time we shall have a more complete and satisfactory knowledge of the main phases of phylogeny and the chief lines of evolution of our higher plants.

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THE GENETIC RELATIONSHIP OF PARASITES[1]

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Parasitism is a mode of life having many forms and degrees. Nutritional dependence may be partial, temporary, or complete. All possible intergradations exist. It has been estimated that a large percentage of the total number of seed plants may use complex food derived from other organisms and yet only a comparatively few assume a mode of life which has been regarded as parasitic. Gradations also connect the organism, which is dependent upon other living organisms for nourishment, with those which are dependent upon organic, but non-living, food.

Organisms may be classified physiologically as parasites, saprophytes, and autophytes (or holophytes) but such a classification does not coincide with a taxonomic one. Upon morphological grounds parasites are variously distributed in the taxonomic series with independent or saprophytic forms. If our taxonomic classifications can be regarded as representing phylogenetic relationships their arrangements not only suggest the evolution of parasites from free-living ancestors but also indicate many separate points of origin. The presence of free stages in the life-cycle of many parasites; the frequent occurrence of temporary parasitism; and the close morphological resemblances between some independent and some dependent forms afford abundant evidence to justify such a view.

Organic dependence may have begun gradually or it is possible in some instances that the time element may not have been a factor and that parasitism may have come about as a sudden mutation. Plausible explanations of the origin of the parasitic habit in animals do not seem so difficult as in plants. There is the possibility that the seeking of shelter may have been the starting point for some animal forms, others may have found protection accidentally having been swallowed in an embryonic or even adult condition. It has been suggested that in many cases the habit may have begun "with the naturally more sluggish females, prompted not by hunger, but by the impulse to seek some conveniently sheltered place for the birth of the young." It may be possible eventually to gain some usable hints from the past ages but parasitic conditions are so dependent upon adaptations in the physiology and soft structures that the record in the rocks is exceedingly fragmentary and difficult to interpret.

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[1] Invitation paper read at the symposium on "The Genetic Relationship of Organisms" before the Botanical Society of America at Philadelphia, December 30, 1914. Contributions from the Department of Botany, Pennsylvania State College, No. 3.

When parasitism is suggested the one feature concerning the parasite which presents itself most strongly is that of degeneration. The dogma of degeneration is so thoroughly drilled into the minds of the students of biology everywhere that parasitism and degeneration seem unalterably linked together. We are prepared for the expressions that parasites "are more or less degenerate according to the extent of their parasitism" or that parasitism "unfailingly involves degeneration." Although we may expect old and popular beliefs to be overthrown at any time there seems to be much in favor of the correctness of this one. So far as the seed plants are concerned there is evidence of somatic degeneration, consisting chiefly of reductions or atrophies of the shoot and root, even in those forms which are only partially parasitic; while those which are fixed parasites may be still further reduced, the root failing to develop, the shoot remaining unbranched, the leaves lacking chlorophyll and frequently appearing as colorless bracts. With regard to the spore plants it is not so easy to form judgments concerning possible somatic modifications since there are likely to be long genetic series of heterophytic forms and no clearly related autophytic groups to form a basis for comparison.

As regards sexuality it has been a widely accepted doctrine that the parasitic mode of life leads to its disappearance. Investigations by Ernst and Schmidt on the root parasite *Rafflesia* have caused them to draw the general theoretic conclusion that so far as seed plants are concerned parasitism does not tend to the disappearance of sexuality. Other studies have, however, shown that the fruits and seeds may show various specializations. In the case of the fungi we are not surprised to find in an authentic text-book, now only fifteen years old, the statement that sexual reproduction is common in the Phycomycetes, occasionally occurs in the Ascomycetes, and is not certainly known to exist in the Basidiomycetes, with the added explanation that probably certain of the higher forms of fungi that have lost their sexual methods of reproduction have been derived from the lower fungi. Recent researches have shown that the old belief in the disappearance of sexuality in the parasitic fungi is no longer tenable. Harper has summed up the situation for the fungi in the statement that "the evidence is now generally accepted that either a typical conjugation of normally differentiated gametes and their nuclei or some form of substitute for it is everywhere present." The very fact, however, that there is here interpolated the phrase "or some form of substitute for it" suggests strongly that although there may not be a disappearance of sexuality there may be a degeneration. In fact the author just quoted states further that the fungi exhibit some fundamental modifications of the process of sexual fusion as found elsewhere. The question whether these variations are to be regarded as degenerations may be debatable but inasmuch as they are considered substitutions for sexual fusions that would appear to be implied. It should be noted here that while sexual reproduction can be shown not to be entirely impossible for certain forms yet its occurrence may be exceedingly rare. Long patient researches with some forms such as the late blight fungus of potatoes have led to an almost inevitable conclusion that there is not a disappearance of sexuality but that there is a loss of sexual vigor. It seems reasonable to conclude that the presence of both somatic and sexual modifications in parasites warrant the continued association of degeneration with parasitism.

In connection with this view that parasitism is a degenerate adaptation it would be interesting to know whether there are any cases where the parasitic habit has been conquered and a return made to a normal independent existence. MacDougal in speaking of the higher plants says that it is usual to consider them "as passing down an inclined plane of atrophies, which would ultimately lead to their extinction, without reference to the abundance of development of the host forms." He has not in his investigations obtained the slightest hint which would indicate the possibility of a retracement by which a parasite might regain its standing as an independent. Nor is there any evidence from the bacteria or fungi to suggest that the parasitic habit when once established ever can be overcome. Some investigators hold the view that certain groups of obligate fungous parasites have made morphological advances of a sporophytic nature but this is questionable and even if true could not be taken as an indication of a possible step toward abandonment of parasitism. Some evidence which seems to point in this direction comes from the animal kingdom and is furnished by a paleontologist. The case referred to is that of the parasitism of the gastropods on the crinoids and has been very clearly set forth by Clarke. Gastropods of the limpet type were parasitic on crinoids from the Silurian on to the latter part of the Paleozoic when all traces of such a condition ceased. The evidence points toward the cessation of this parasitic condition during Carboniferous ages. Clarke believes "that all the positive and all the negative evidence we can now adduce on this deeply important subject favors the presumption that the habit was abandoned or at least, to speak in terms of simple casuistry, was lost." There are no parasitic snails on crinoids today, but close allies of the crinoids are parasitized by gastropods of the limpet type. This, however, is held to be entirely independent of the former association between these groups.

Much has been written concerning the nature and origination of parasitism but much remains for further experimentation before the factors involved can be understood. In order to form proper conceptions it is necessary not only to consider the parasite and its weapons, but also the host with its defense, so that the question of the origin and nature of parasites is inseparably associated with those phenomena generally grouped under the term immunity. We must take into account also the effects of the environment upon both parasite and host. The available evidence indicates such a difference in the genetic physiology of parasites belonging to the higher and lower plants, respectively, that it may be best to discuss the groups separately.

The parasitic seed plants form a series of isolated groups each of which bears a more or less evident genetic relationship to some independent group. Any theory of origination must take into account the undoubted existence of a number of disconnected points or levels of origin. MacDougal has extensively investigated the conditions under which two plants may enter into the relation of host and parasite by growing one plant upon another and has concluded that "the ruling factor was in all cases the osmotic ratio between the sap of the two plants," a greater osmotic pressure being necessary in the incipient parasite. Of course many other factors are involved for one plant is not able to parasitize another simply because it possesses a higher osmotic pressure. It seems likely that parasites among the seed plants arose directly from independent ancestors without any intervening saprophytic condition such as probably existed in the evolution of the fungi. The first step intervening between independence and dependence in the seed plants may have been the absorption of water and food materials, the taking up of complex foods being a later development. This is in accord with a theory hazarded by Cowles who adds that as a final development there comes the loss by the seeds of the power to renew their growth, except when in contact with the host, and the development of a univorous habit.

As intimated in the foregoing paragraph there is evidence indicating that the parasitic lower plants (bacteria and fungi) have developed from independent plants not directly but through intermediate saprophytism. There are numerous transitional stages from autophytes to saprophytes and on to facultative parasites, ending finally in obligate parasitism. The development of the capacity to take in organic foods in a saprophytic manner seems to be a simpler first step for these forms, rather than the absorption of water and food materials in a parasitic way, and at the same time the existing forms grade into one another along such a probable course of development. Even after parasitism is attained there are still degrees of differentiation. The forms which can continue without sufficient detriment to the host to cause its death, and hence their own, are to be regarded as on a higher plane of development than those whose aggressive growth soon results in death for both host and parasite. Another condition which is agreed by many to indicate a high parasitic evolution is the physiological dependence upon a very restricted set of hosts. The greater the restriction, which may be a single host species, or even a variety, the higher the stage of parasitic development.

Concerning the independent forms which may be looked upon as most nearly representing the probable ancestors of such forms as the parasitic bacteria and fungi there is general agreement in a broad way but naturally differences of opinion upon many important points. The probable connection between some dependent and independent forms has received definite recognition in the classifications used by some taxonomists. Bessey no longer maintains the Phycomycetes co-ordinate with the groups of higher fungi but places them in the phylum with the tube algae upon morphological grounds. In like manner the Synchitriaceae are grouped with the unicellular green algae. The series classed as higher fungi are more isolated and retain standing as a separate phylum with debate still going on as to their relationships and interrelationships.

In a recent paper Jones has emphasized the fact that the fundamental problems of parasitism remain unsolved, that "we have scarcely begun the study of the intimate relations of parasite and host, the conditions and results of parasitism." He comments further that the very simplicity of the plant's organization makes these relations more difficult to investigate than with the animal, the cell being the unit, not the organism, the study of chemical interrelations is rendered highly difficult. "Such problems," he says, "call for the combined skill of pathologist, physiologist, cytologist, and chemist." These forces, however, have not been mobilized adequately as yet.

Chemotropism is frequently put forward as a factor involved in the origin of parasitism in the lower plants. Massee goes so far as to assert infection is due to positive chemotaxis. If it is the pulling power of some substance within a host which causes fungi to enter, it is difficult to explain highly restricted parasites. Either very few hosts contain the proper substance or all others must contain something to neutralize this attraction or something of a more powerful repellant nature. Neither view seems adequate to give us a conception of a large percentage of the world of plants with parasites more or less specialized to themselves. It must be kept in mind also that mere entrance of a fungus is only preliminary and not necessarily indicative of parasitism. The theories of immunity come to mind, pass in review, and leave us unsatisfied. One investigator states that "immunity depends chiefly (perhaps entirely) upon the ability of the cytoplasm of the host-cells to resist infection by secreting anti-toxins which will kill the mycelium of the fungus." Tannin, vegetable acids, and oxidizing enzymes have been named as possible toxic substances. Others would not place the burden of keeping invaders out upon the cell-contents of the host, but explain the failure of parasites to grow promiscuously upon their lack of development of cytolytic enzymes sufficient to break down the barrier offered by the cell wall. It makes little difference which of the theories has the most supporters or which one appears most plausible; not one of them, or any combination of them, gets at the real intimate relation which must exist between host and parasite. Chemical, food, or structural features, as far as we are able to appreciate them, seem unable to explain why one parasite can attack only one variety of a single host species while another similar parasite may attack all the varieties, other species of the genus, and perhaps even extend to other closely related genera.

Our problem would be sufficiently baffling if we were left to consider only the relation between a parasite and its host, or hosts when it passes to several allied forms, as indicated in the foregoing paragraph. No matter how difficult this may appear to be we are obliged to designate this as simple parasitism. To contrast with this there are the cases of parasites which are known to inhabit wholly different and unlike hosts in different stages of their life histories. Such a surprising and, as it seems, unnecessarily complex parasitism exists in both animal and vegetable kingdoms. In those species which change hosts, and on that account are termed heteroecious, there is frequently more than one method of propagation. Protozoan forms which come under this category are said to multiply by schizogony, a kind of multiple fission, and by sporogony, a process of spore-formation preceded by conjugation. Sporogony usually either makes possible the invasion of a new host or takes place only in connection with the second or intermediate host. The evident food relations between the hosts of many of the heteroecious animal parasites, such as the mosquito and man and various mammals in the case of malarial forms, renders the association of the unlike hosts not so difficult to appreciate.

In the higher fungi, especially in the group popularly known as the rusts (Uredinales), there are many instances of heteroecious parasitism and in none of them is there the slightest hint of a relationship between the two hosts. There are many forms inhabiting coniferous trees and from these they may pass over in their alternate stage to plants widely separated in taxonomic classification. Thus we may find a form alternating between a fir and a fern, a cedar and an apple, or a pine and a composite. It seems impossible to escape the conviction that some extraordinary relationship must exist between these two sets of hosts occupied by a heteroecious form and yet in the half-century since we have had definite proof of heteroecism in the rusts no satisfactory explanation has been forthcoming. In North America we have today almost exactly one hundred demonstrated cases of heteroecious life-cycles in this group. The rusts are of especial interest from our present point of view not only because of heteroecism but also because of the existence of autoecism within the same group, and the consequent opportunity to compare these two types of adaptation. Heteroecious forms always possess what may be termed a long life-cycle divided necessarily into two phases, on the one host consisting of telia, either alone or accompanied by uredinia, and on the other host of aecia, preceded by pycnia. Telia, uredinia, and aecia produce spores usually unlike one another and are all for purposes of propagation. Pycnia produce simple spores unlike any of the others and of unknown function. Autoecious forms may exhibit the same number of spore-forms and in the same sequence as just described for heteroecious forms, or they may have the aecia and uredinia lacking. In this case the pycnia, when present, are followed at once by telia and we have what may well be designated as a short life-cycle. The heteroecious forms always show well developed pleomorphy whereas the autoecious forms may or may not. The presence or absence of urediniospores, which simply repeat themselves over and over, is held to be of no fundamental importance in the formation of a life-cycle. It is understood that telia are always present and that pycnia usually (but not always) follow from telial infection. If a heteromorphic spore-stage (aecia) follows the pycnia the form is thrown into the long-cycle series without regard to the presence or absence of a second heteromorphic stage (uredinia).

Ever since heteroecism has been definitely known to exist in this group, or for fifty years, naturally there have been speculations concerning its origination. Within the last ten years since sexuality and an alternation of generations have been demonstrated the character of the speculations has been somewhat modified. The probable evolutionary tendencies within such a remarkable group of obligate parasites must have an important bearing upon our general conception of the genetic physiology of parasitism.

If we accept a view, which has found much favor, that the complex heteroecious types have been derived by amplification from the simple, autoecious, short-cycle forms, we then find it necessary to explain how parasites, in which degeneration might be expected, have made unprecedented advances. To be sure it seems most logical to derive the complex from the simple and the amplification, since it is in the sporophytic stage, is in accord with the conditions found in independent plants. But the questions immediately arise whether we can expect such obligate parasites to parallel the development of higher plants and whether we need to make such assumptions in order to explain present conditions. In the light of our observations on the behavior of parasites it seems that we ask a good deal of both evidence and probability in seeking to align these parasites with the independent higher plants on the basis of the development of the alternating generations.

Both short- and long-cycle forms agree in the gametophytic generation which is initiated by the reducing germination of the teliospore and ended by a doubling conjugation previous to the production of the first spore-structure (the pycnia being disregarded). In the short-cycle condition the first spore-structure is another telial stage hence the sporophyte is represented only by the teliospores themselves and a small amount of growth accessory to their production. In the long-cycle condition the first spore-structure following the conjugation may be known as the aecial stage without regard to its form or structure. In all cases the aeciospores give rise to a plant body (mycelium) which is sporophytic. In some forms it produces repeating spores, either like or unlike the aeciospores, but ultimately forms the telial stage, the end of the sporophytic phase. In heteroecious forms the two phases are produced upon the unlike hosts. Reasoning by analogy we are justified in regarding the gametophyte as the primitive generation and it would seem to follow that the forms with the least developed sporophyte should be nearest the ancestral conditions. By a progressive development of the sporophyte, a prolongation of the double nucleated phase, we have an evolution which is comparable with the course of development of the higher plants. Some investigators have become so confident that this is a reasonable presumption that they have ceased to argue this phase of the problem but have gone on to discuss the origin of heteroecism, how the jump from autoecism to a new host could have come about, and which was the original gametophytic host and which the secondary one.

If the rusts have made such advances in their sporophytic generation we have a condition which may force us to modify our ideas of parasitism. The whole question is inseparably connected with the large problem of the genetic relationship of parasites. The question whether this group with all its complexity conforms to the nature and development of other parasites or stands as an exception is one which interests us today.

Those who have regarded the rusts, in spite of their complexity, as a degenerate group of parasites also have advanced theories as to the probable course of development. It may be idle to speculate further with such theories but perhaps another viewpoint may permit new relations to be perceived. According to one view pleomorphy and even heteroecism are held to be the primitive condition and the autoecious short-cycle forms are regarded as the latest in evolution having arisen by a process of degeneration and reduction. It is not denied that there must have been amplification some time in order to attain the long-cycle condition but it is believed that this did not take place after those organisms became parasites. Undoubtedly the long-cycle forms represent the highest development but probably not the latest. As suggested in a foregoing paragraph there is much in favor of the parasitic fungi having passed through an intermediate saprophytic stage. Ultimately, of course, they must have come from independent ancestors. The existence of independent thallophytes and saprophytes showing well developed pleomorphy indicates that this feature is not one which can be considered as having any peculiar connection with the parasitic mode of life. The transition of complex pleomorphic forms into heteroecious parasites may not seem an easy step but is not more difficult to appreciate than the interpolation of one or two new stages, upon an entirely foreign host, into the life-cycle of a simple parasite.

Since our present interpretation of the evolution of the higher plants is the rise of the sporophyte and the decline of the gametophyte much has been made of an analogical application to these parasites. It has been pointed out that those forms with the longest sporophytic stages must have arisen in a progressive way. While this is doubtless the case there is much doubt whether they have progressed as parasites and whether the present short-cycle forms can be looked upon as representing their ancestors. It should be pointed out that there has been no decline of the gametophyte unless it be in the loss of pycnia in some species. However, the only forms in which pycnia are known to be absent are short-cycle forms, which are according to the theory of ancestral nature. The pycnia are without doubt functionless today. It would be odd indeed if the gametophyte of the latest type in evolutionary history should always develop a useless structure not universally known in its ancestral forms. Surely this is not in conformity with the higher plants. There has been much discussion over the interpretation of the pycnia in the rusts. The view that they represent vestigial male organs is highly plausible. Without much doubt they have not been functional since the parasitic adaptation. A former sexuality in which an Aecidium-like structure bore the female organs is now replaced by a somatic doubling conjugation in the mycelial mass below the spore-structure. The eventual disappearance of the pycnial stage might be anticipated in the latest forms. If this has any bearing it points toward the short-cycle autoecious type as the most recent for here the pycnia may be omitted although in ontogeny there elapses a time for their development.

In a parasitic group where there is such a close relation between parasite and host we might gain some idea of genetic relationship from a study of the distribution of the different types of rusts upon the series of hosts. Although the phylogeny of the hosts may not be regarded as settled the arrangement will permit this sort of a general comparison. It seems logical to assume that the lowest hosts would be parasitized by the more primitive forms while the higher host orders would be expected to have upon them the more advanced types. As already indicated we recognize but three types of parasites, heteroecious long-cycle, autoecious long-cycle, and autoecious short-cycle. The ferns are the lowest hosts for these parasites and their forms are pleomorphic and heteroecious. In order to have some numbers available a careful survey of North American forms having a definite known life-cycle has been made. Out of fifty species on Coniferales forty-seven are heteroecious. Glumaceous rusts are heteroecious with a single possible exception. In the Lily order while only seven are heteroecious, twenty-three are autoecious long-cycle forms, with a single short-cycle form. The Ranales have the three types almost equally distributed. In the large rose order the long-cycle forms greatly predominate but autoecism is three times more prevalent than heteroecism. Upon the final great alliance, Campanulales, we find heteroecious phases but so far as present known life-cycles are concerned they are only half as numerous as short-cycle forms, the figures being thirty-two and sixty. If we make comparisons within the short-cycle series we find that they are almost unknown upon the lower orders whereas sixty out of a total of one hundred forty-eight known forms are found upon the highest order. If this sort of evidence is competent its indication is certainly plain.

In a recent paper Olive has taken it for granted that autoecism is the original condition and has brought forward arguments bearing on the question as to which of the heteroecious hosts is the primary one. With his premises as a basis he comes to the very logical conclusion that the present aecial host was the original host of the hypothetical autoecious ancestor. Part of the argument is that the gametophytic generation being the more primitive should be on the primary host. To evolve the heteroecious condition the gametophyte has remained unchanged but the sexual fusions have furnished the invigorating influences which are responsible for pleomorphy and the pleophagic habit. It cannot be denied that sexual fusions should furnish invigoration but those fusions found here which appear to be a degenerate substitution for a former fertilization do not seem adequate to account for such a profound change in the life of a parasite.

If we are to take the view that the present aecial host is the original and primary one, then it would seem reasonable to expect the aecial stages to be on the more primitive hosts as a rule. The condition found in our North American heteroecious forms does not bear out this expectation for we find out of ninety-nine species that seventy-eight have aecial hosts standing higher in classification than their telial hosts. To support the argument that the gametophytic host was the original autoecious one, it has been urged that there are instances where short-cycle forms are known on the aecial hosts of heteroecious species, the teliospores of the two showing structural parallelisms so striking that some kind of relationship is strongly suggested. *Puccinia mesnieriana* a short-cycle form occurring on *Rhamnus* and having teliospores peculiarly like *Puccinia coronata*, a heteroecious grass form having its gametophytic stage on *Rhamnus*, has been cited. There are numerous other cases. *Puccinia Xanthii* is an autoecious form on *Xanthium*, which harbors the aecial stage of a sedge rust, *Puccinia Cyperi*. *Polythelis fusca*, a short-cycle form on *Anemone*, has teliospores identical with *Tranzschelia punctata* which has its telia on *Prunus* and its aecia on *Anemone*. Numerous other cases could be mentioned. May the evident genetic relationship best be explained by amplification or reduction? Not only does the latter more nearly conform to the physiology of parasitism but also agrees with certain conceptions of differentiation which have been formed from experimental genetics. Based upon many considerations we find Bateson saying that he feels no reasonable doubt that we may have to forego a claim to variations by addition of factors, yet variation by loss and fractionation of factors is a genuine phenomenon of contemporary nature. If there is a possibility that we may have to dispense with additions from without in independent forms how much more strongly does such a situation press its claim for recognition in parasites. To return briefly to the associated long- and short-cycle forms above mentioned we find that no startling variation has to be assumed in order to derive the latter from the former. If on the gametophytic host, following the sexual fusion, there should be produced at once teliospores instead of an intercalation of one or more polymorphic spore-stages, the proper conditions for a short-cycle autoecious form would be at once established. In heteroecious forms only the reduced basidiospores following teliospore germination can infect the aecial host. In the abbreviated condition just outlined we have the necessary requirements for reinfection and maintenance of a short-cycle telial form on what was the original aecial host. Believing that the only fundamental stages are those of sexual fusion and teliospore formation neither is disturbed by such a shortening process. Teliospore formation which was an inherent capacity simply takes place sooner than formerly without the intervention of a somatic stage of such extent between sexual fusion and reduction. If teliospore production is to take the place of the former aeciospore formation there might be two expectations concerning their nature. They might be exactly like the former teliospores in which case nothing happens except the pulling of the stage backward to the other host, or they might be expected to have the morphology of the aecidiospores but behave physiologically like teliospores. The so-called genus *Endophyllum* fits the latter hypothesis and may have arisen in some such manner. The cases cited and numerous others fit the former. Such a theory does not fit badly with the present distribution of the types of rusts upon the phylogenetic series of host plants. Recalling the fact that the tendency for heteroecious species is to have the gametophytic hosts higher in classification than the telial hosts we must expect the short-cycle forms which are to occupy the former gametophytic hosts to show an upward movement which would result in their occupation of the higher orders. I have already shown that is in accord with the facts based upon a survey of North American forms. Although it may be objected that these considerations do not explain the real origin of the group it may be claimed that they do express a trend of evolution, since a time when the group attained a parasitic mode of life, which accords with the course of development everywhere bound up with parasitic adaptations.

Someone has said that nature produces individuals while man has created species. This seems hardly a proper observation for higher plants and animals but it applies well to the lower parasites. The fact that we hear of "physiological" species, "biologic" races, and "formae specialis," in parasites is but an admission that man is the inventor of "species" of some groups and in order not to be misinterpreted finds it necessary to qualify the term. Cook and Swingle have pointed out that "normal and long-sustained evolutionary progress is not accomplished on single or narrow lines of descent, but is possible only for large companies of interbreeding individuals; that is to say, for species." Certainly in all organisms where we have interbreeding and interweaving of distinct lines of descent there is such a thing as a real specific group but in the parasitic fungi all traces of the coherence of such a group has been lost. With the exception of the mucors, where the interweaving of strains is definitely known, and the possible occurrence of sexual strains in some downy mildews and anthracnoses, there is no suggestion of groups of interbreeding individuals. The individuals continue their existence without relation to other individuals. It is clear that man makes the species here solely for his convenience and he must decide whether he will make morphology or physiology his standard. If it is true that progress cannot be maintained along narrow lines of descent this will assist in understanding why advances are not to be found in the parasitic fungi for here the lines are the narrowest possible, simply the continuations of individual existences. If we are given an individual spore we can start a life-cycle which will bring us back to more spores of the same sort and we may raise generation after generation but they are descendants of a pure line in no way influenced by individuals of different origins. The factors which may influence them are their hosts and the environment.

Through morphology we form our judgment concerning the possible similar origin of individuals which come to us to classify. In the parasitic fungi as a rule it is only the reproductive features that receive morphological consideration. If we considered only dead specimens there would be no difficulty, but with the advent of experimental culture work, perplexing questions have arisen. The work of Eriksson, Klebahn, and others served to emphasize this matter but it is clear from DeBary's writings that he was aware of the specialization of a parasite to a host although he did not use the form of expression now prevalent. Morphological equivalents growing upon the closely allied hosts may not have the same behavior. The powdery mildews, anthracnoses and rusts furnish many familiar instances. This means that the plant body is coming in for attention and that the consideration must be of a physiological nature. The question whether forms structurally alike but reacting physiologically differently should be regarded as belonging to the same species or a race, etc., may be an interesting one but is not the most important one. The bacteria are notable organisms which raise such questions. But the genetic relationship is of greater significance. It is a common view that the forms morphologically alike must have had a common origin and that physiologically specialized forms have resulted by progressive variability on a special host. Nutritional adjustment and other interacting factors have been suggested as associated with the variations but no demonstration up to the present has made the matter at all clear. It has been assumed that continual specialization will lead eventually to structural differentiations. There seems to be nothing in the methods of descent which would prevent the carrying forward of any variation once established. It is rather a question of how the physiological relations between host and parasite or the environment might operate to bring about a diversity of form and structure.

Very little is known of the possible effect of the ordinary factors of environment upon the development of a parasite. There is some evidence regarding effects upon distribution. After a detailed comparison of the geographical distribution of hosts and parasites in one group of fungi, the writer came to the conclusion that the environment had little if any influence at all, but such apparently is not true for all kinds of parasites. Ward has shown that external influences may undoubtedly exert important effects. Too high a temperature during incubation, starving the host of carbon dioxide or salts, or heating or cooling the roots may ruin a fungus and stop infection. Salmon has also carried out investigations to show that external as well as internal features are concerned in determining whether parasites may attack and continue development.

As a conclusion we may consider, with brevity, parasites as an aid in determining genetic relationships in their hosts. Cobb has written on this subject in a very enthusiastic way, pointing out that parasites may be used (1) as an aid in discovering specific and generic relationships, (2) in following metamorphoses, (3) in physiology, (4) in chemistry. I am particularly interested in the first of these. I trust I may be pardoned for drawing my examples from a group in which I have long had a special interest. A recently published result brings this phase of the subject to mind. The members of the genus *Gymnosporangium* were long supposed to alternate solely between the juniper and apple families. Upon the morphology of a spore-form found upon a member of the rose family I suspected its connection to a cedar rust and assisted in proving the relationship. Later a similar case was discovered in the hydrangea family and it, too, was established. The Malaceae, Rosaceae, and Hydrangeaceae have always been regarded as close relatives, being included in the order Rosales. Recently Fromme has reported a fourth family, Myricaceae, as furnishing a host for a *Gymnosporangium*. Since the other three families are evidently related, there is a strong suggestion that this one should stand nearby in the scale of classification. In Engler and Prantl's system it is, however, in the fourth order of the Dicotyledoneae while the Rosales is the twenty-first order. The Myricales order has a similar low position in the latest manuals of Gray and Britton. It is interesting to note that Bessey, on the other hand, places the family Myricaceae in the order Sapindales which he derives from the Rosales through the Celastrales. The latter arrangement is much more strongly supported by the parasites than the former. In this same group of fungi there is some evidence regarding the relationship of the other series of hosts. It is generally accepted that the § *Oxycedrus*, of the genus *Juniperus*, with its subulate leaves, is of earlier origin and probably ancestral to the § *Sabina*, which has its leaves subulate when young but scale-like when mature. The species of the § *Oxycedrus* are distributed in both hemispheres and appear to be identical. Species of the § *Sabina* are widely distributed but certain species are indigenous to one hemisphere and certain ones to the other, there is not one that is common to both. The parasites of the genus *Gymnosporangium* parallel the condition of the hosts exactly. We do not find the same species indigenous to both hemispheres on the § *Sabina* but we do on the commonly distributed section. The distribution of the hosts would suggest separate origins of the derived section in North America and the Old World and this theory is strongly supported by the condition of the parasites. These examples will suffice to illustrate the matter. Our knowledge is too incomplete to determine the real value of this phase of the subject and yet we may conclude that a knowledge of the parasites is worthy of consideration in a study of the hosts.

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THE EXPERIMENTAL STUDY OF GENETIC RELATIONSHIPS[1]

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The earliest experiment designed to determine the genetic relationships of a plant was carried out by Linnaeus and recorded in his famous disquisition on the sex of plants. He tells us that late in the autumn of 1757 he stumbled upon some plants of *Tragopogon hybridus* in the botanic garden at Upsala, growing in a bed where only *Tragopogon pratensis* and *Tragopogon porrifolius* had been planted. Before this time, as we know from Hartmann's dissertation on hybrid plants, Linnaeus had thought it possible that *Tragopogon hybridus* was a cross between *Tragopogon porrifolius* and *Lapsana stellata*—species of two quite unrelated genera. Now, however, he had a clue to its true relationship, which he set about to prove. The next year he removed the pollen from some heads of the yellow-flowered *Tragopogon pratensis* and sprinkled the styles with pollen from the purple-flowered *T. porrifolius*. The hybrid seeds thus obtained were planted in the fall and gave plants which were found to be identical with *Tragopogon hybridus*. The flowers were not completely purple, as in the staminate parent, but showed the influence of the pistillate parent in their yellow bases. After this experiment, Linnaeus tells us, it was impossible to doubt that new species might come into being by hybridization. His conception had changed greatly since the publication in Fundamenta Botanica (1735) of the oft-quoted dictum, "*Species tot numeramus, quot diversae formae in principio sunt creatae.*" (Every species which we can enumerate was created in the beginning a distinct form.)

The more mature views of Linnaeus, however, were in advance of his time. Philosophia Botanica (1737), in which the doctrine of special creation was set forth, continued, long after his death, to be the *vade mecum* of botanists. The conception of the hybrid origin of species was so completely disregarded that Sir James Edward Smith, an ardent Linnaean, the possessor of the Linnaean herbarium and founder of the Linnaean Society, wrote in 1807, "By species are understood so many individuals, or, among the generality of animals, so many pairs, as are presumed to have been formed at the creation, and have been perpetuated ever since; for though some animals appear to have been exterminated, we have no reason to suspect any new species has been produced; neither have we any cause to suppose any species of plant has been lost, or any new one permanently established, since their first formation, notwithstanding the speculations of some philosophers."

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[1] Invitation paper read at the symposium on "The Genetic Relationship of Organisms" before the Botanical Society of America at Philadelphia, December 30, 1914.

Although Linnaeus had reached the remarkable conclusion that species were, in some way, genetically interrelated, the second edition of the "Species Plantarum" and the inaugural dissertation of his pupil, Hartmann, contain so many preposterous guesses as to the parentage of certain supposed hybrid species, that he could certainly have had no conception of the limits of hybridization, or of the segregation which so often follows it. Obviously, he supposed that species originated as stable first generation hybrids—not a surprising theory, considering that, in his experiments with *Tragopogon*, he had chanced upon the one family in which parthenogenesis is so common that hybrids are often constant from generation to generation.

Linnaeus, knowing nothing about the geological history of organisms, had to construct a scheme of evolution which involved only the Mosaic "days of creation" and the few thousand years of recorded history. Having assumed the hybrid origin of species, it was clearly necessary for him to hypothecate the creation in the beginning of a considerable number of distinct forms representing the natural orders, for otherwise there could have been no material for hybridization to work upon. Moreover, since these originally created forms were so different that one could hardly believe in their crossing, he invoked miraculous intervention to account for it. Finally, in order to preserve intact the limits of the orders, he seems to have implied that the more fundamental characters were transmitted by the mother; the superficial ones by the father. His philosophical difficulties and the way he got around them now seem very amusing; but, as we shall see later, the same difficulties still confront certain modern geneticists, who have either not been able to circumvent them at all or are beginning to whisper a hypothesis more fantastic than that of Linnaeus.

Although Sir James Edward Smith's views were those which prevailed, for the most part, until the time of Darwin, there were occasional outcroppings of evolutionary ideas. On the botanical side, the views of Dean Herbert and of Naudin were based largely upon experimentation rather than speculation and were expressed with especial clearness. The former (1822 and 1837) said that "horticultural experiments have established beyond the possibility of refutation, that botanical species are only a higher and more permanent class of varieties." The latter (1852) said that the methods by which the gardener produces new varieties coincide with the processes by which new species originate in nature. It remained for Darwin, however, to refute finally the doctrine of special creation.

Darwin ascribed the origin of new species in nature to the natural selection of favorable variations. With this view, in its most general aspect, nearly everyone is in accord. But the great problem of evolution still remains,—what is the source of the variations which are selected? Darwin could not answer this problem satisfactorily because no one had yet discovered the distinction between fluctuating variations, which are not inherited, and mutations, or germinal variations, which are inherited. He also went astray, as we now think, in believing that the effect of use or disuse of an organ could in some way impress itself upon the germ-plasm and become hereditary. The few who still hold that the selection of continuous variations would suffice to bring about specific differentiation can bring forward little or no evidence to support their view. The evidence all points to the utmost fixity of organisms, aside from mutations. In order not to perpetuate a misrepresentation of Darwin's views which he himself particularly resented, it should be said that, after the publication of the Origin of Species, Darwin came to believe that he had formerly underrated the value of mutations ("spontaneous variations") in bringing about diversity. Even in the first edition of his great work he stated his belief that the selection of insensible derivations had not been the exclusive means of modification. Darwin's caution, however, was not shared by all of his followers. Exaggeration and misrepresentation of his views led to an almost universal conviction that modification was too slow a process to be made the subject of experimental inquiry. Thus it came about that the Origin of Species was followed by a period of stagnation, as far as experimentation was concerned.

During this period there were indeed a few experimenters, with the courage of their convictions, who carried on genetical studies. One of them was Mendel, whose investigations aroused no interest among his contemporaries, although they were stated with unsurpassed clearness, and would have changed the current of biological thought if anyone had realized their bearing on the theory of evolution. Another was Focke, who laid emphasis upon the importance of hybridization in species formation. Even then, as now, he believed that, in such large and polymorphic genera as Rubus and Rosa, many of the species have originated by hybridization, followed by the sorting out of stable forms.

From Darwin's time until very recently, however, it has been the prevailing view that the selection of extremes from a continuously varying population would result in a continuous, gradual modification of the entire population, and that such selection had brought about the formation of varieties in cultivation and of species in nature by the simultaneous transformation of masses of individuals. It need only be said that carefully planned and executed experiments lend no support to this view. All the evidence, on the contrary, seems to show that no amount of selection will suffice to modify the range of fluctuating variation of an organism. On the botanical side there is little evidence of the efficacy of selection as a factor in evolution; on the zoölogical side there are certain selection experiments of Castle's, carried out with characteristic care and accuracy, but surely capable of a different explanation from that which he gives. Even if correctly interpreted they have at best a dubious bearing on the problem of species formation. His experiments deal with the inheritance of a certain color pattern in rats, which, in a presumably homozygous race, may be modified in either direction by selection. The changes from generation to generation are very slight, however, and we cannot conceive of any agency in nature which would bring about assortive mating among such slightly dissimilar individuals. While it is becoming increasingly clear that the old selection theory is untenable, we are becoming more and more convinced that evolution does take place with measurable rapidity, and that the factors concerned with it are mutation and hybridization.

The new point of view we owe primarily to de Vries, who has determined the distinction between non-heritable fluctuating variations and inheritable germinal variations, or mutations, and has developed the mutation theory. It should appeal especially strongly to systematists, most of whom have really never been convinced of the adequacy of the discarded selection hypothesis. Why, if species had come about by such a gradual process, were not intergradations more common? Why did most of the supposed intergradations prove to be rare and partially sterile hybrids? Why, if evolution were still going on, could one recognize speciation at all in some of the groups? To the theoretical evolutionist, who knew as little as possible about species, such questions indicated merely the perversity of the systematist. The latter, accepting the general truth of evolution, but influenced hardly at all in his attitude by the manner in which it was supposed to have taken place, continued to describe species and then still more species, just as he had always done, and just as he will doubtless continue to do. Sometimes he told what he thought about their relationships, oftener he did not, but he seldom failed to add to his description some variation of the formula "entirely distinct from its nearest ally." He has been anathematized; some of his colleagues have even threatened to cast him into outer darkness. Nevertheless, his work has certainly been as truthful and as serviceable as the work of those who deplore his "raking together of straws and sticks and even antique dust."

It will require the combined efforts of morphologist, systematist, and geneticist to arrive at the whole truth in regard to genetic relationships. In one way the geneticist has a great advantage over the other workers, for his methods are inductive, whereas theirs are deductive. In another way he is at a great disadvantage, for he can deal only with the lesser categories, the variety, the species and perhaps the genus. The relationship of the larger groups must be determined, if at all, by the deductive methods of the morphologist, and, I may add, of the biochemist; for, as the years go on, biochemistry will come to be applied more and more to the elucidation of genetical problems.

The immediate aims of the geneticist are (1) to observe the origin of new and distinct forms, the genetic relationship of which must, therefore, of necessity be known, (2) to determine the conditions which brought these forms about, and (3) to study their hereditary behavior and their morphological and chemical characteristics in order to provide a basis for sound deduction in regard to multitudes of types which we can never hope to know except as facts of nature. All three aims have already been realized in some measure as a result of the recent activity in genetics.

Most of the new forms of which the origin has been observed, belong to one class of organisms—namely, recessive Mendelian varieties. Such varieties have been observed to originate in two ways, by mutation, and by segregation from hybrids. Certain ultra-Mendelians maintain that only the latter method is operative, but their argument, which will be discussed after certain examples have been called to mind, can be supported by little or no evidence. As examples of recessive mutations, one naturally thinks first of the remarkable series of 150 which Morgan has observed to originate from the fruit fly, *Drosophila ampelophila*. All of them are recessive to the typical form. On the botanical side there is no correspondingly complex series of recessives known, of which all the members have originated under scientifically controlled conditions, but there is hardly the least doubt that the cases of the sweet pea (*Lathyrus odoratus*), of *Primula obconica* and of *Primula sinensis* are quite comparable with that of *Drosophila*. Some members of each of these large series of forms are known to have come about as mutations, and probably all did. Bateson, who has devoted especial attention to the sweet pea, is sure that all the varieties are recessive to the wild prototype, *Lathyrus odoratus*. It seems sufficiently well proved that recent hybridization has not modified *Lathyrus odoratus*, for no other plant has been found which will cross with it.

In nature there are many examples of species and varieties which bear simple Mendelian relationship to one another. Such a case has been discovered by Trow in the series of elementary species comprehended under the name *Senecio vulgaris*. It also appears that in *Antirrhinum* there are species which seem to be very unlike, nevertheless all of the differences between them can be determined by Mendelian analysis. A large number of stable forms segregate from a hybrid between two such species, and these forms are themselves indistinguishable from species. Their fertility is unimpaired. None of them contains a single character which is not identical with, or recessive to, a corresponding character in one or the other of the parents. Baur and Lotsy have studied the hybrids of *Antirrhinum molle* and *A. majus* from a Mendelian standpoint. The latter has been so impressed by the results that he has come to believe that there is no source of variation in nature except hybridization followed by segregation; it seems to him the sole method of species formation. He explains the so-called recessive mutations, no matter how rare they may be, by the assumption of previous hybridization and a sufficiently large number of multiple factors. It seems fair to ask the holder of this view how the forms originate which supply the characters to be assembled and reassorted by hybridization. This question brings him face to face with the philosophical difficulties of Linnaeus. Either he must deny evolution, or, with Bateson, confess that the studies carried on by Mendelians have thrown no light on the problems of evolution. Bateson suggests that we should seriously consider the possibility that evolution has not taken place from the simple to the complex, but rather from the complex to the simple; that the original forms of life were heterozygous with regard to all the characters which have ever appeared in geological history; that for each character there was a corresponding inhibitor, and that the characters have come successively to light by the segregation of recessives from which the inhibitors have fallen away. We cannot believe, from the tentative way in which Bateson proposes this fantastic hypothesis, that he really places much faith in it. But the mere fact that he should whisper it shows the extreme pessimism of the ultra-Mendelian attitude in regard to the problem of evolution. Other experimental workers, however, are more optimistic. The Mendelians have been so firmly convinced that differences between species were all capable of Mendelian analysis that they have disregarded facts which did not fit their formulations. Professor de Vries' work is set aside with the statement that the chief reason why factorial analysis has been declared to be inapplicable to the Oenothera mutations is because no one (except Heribert-Nilsson) has set about such an analysis in the right way.

Even those of us who doubt the universality of the Mendelian phenomena, see no reason to deny that species formation by hybridization and subsequent segregation has taken place on a large scale in many groups. One may, however, admit the great prevalence of hybridization, without believing that all variation which may take place subsequently to hybridization is a result of that hybridization. In other words, there is no reason why true mutations should not occur in hybrids as well as in pure lines.

In this connection we may examine a little more closely the view that recessives always originate by segregation rather than by mutation, a hypothesis ancillary to the multiple factor hypothesis. In only one case among wild plants has it been satisfactorily shown that a recessive mutation differs from the parent form in the lack of duplicate factors. This is the case of *Capsella Heegeri* and *Capsella Bursa-pastoris*, which has recently been studied by Shull. He finds that there is a difference of two duplicate factors between the derivative and the parent form. The presence of either factor suffices to give the capsule the triangular shape typical of Capsella. *C. Heegeri*, the recessive, has a capsule shaped like that of Camelina, so that it really departs from the parent form in a generic character. As Solms-Laubach has shown, no systematist who did not know of its mutative origin or of its relationship as determined by crossing would place it in the genus Capsella. All the plants in existence have descended from one individual which was found by Professor Heeger in a colony of *Capsella Bursa-pastoris* in the market-place at Landau, Germany. When the two species are crossed the first hybrid generation is, of course, uniform and like *Capsella Bursa-pastoris*. In the second generation there is one plant of *C. Heegeri* in 16. The 15 sister plants all look alike, but are in reality of three kinds. In the third generation seven will remain constant, four will give one plant of *C. Heegeri* in 4, and four will give one plant of *C. Heegeri* in 16. As far as experimental evidence can be applied it shows that a plant into which the character of *C. Heegeri* has been introduced by hybridization must either give rise to *C. Heegeri* not less than once in 16 times, or not at all. Most recessives, if crossed with the parent form, reappear in the second hybrid generation in a typical 1 : 3 ratio. If they originated according to the multiple factor hypothesis we should have to assume that factors which had been independent for countless generations could suddenly become indissolubly associated in the generation in which the pure recessive appeared. There is not the least evidence that this takes place. Now that we have cases in which segregation in the second hybrid generation occurs in the ratios 15 : 1 and 63 : 1, we naturally expect that some of the recessives which are supposed to depend upon the concurrent absence of so many characters would not reappear at all when recrossed with their parents. With as few as six independent identical factors there would be only one recessive in a second hybrid generation consisting of 4,096 individuals. In a case like that of *Capsella Heegeri* it would be necessary to postulate more characters than six to account for a non-mutative appearance so seldom that only one individual has ever been observed. On the whole there seems no reason to doubt that the sporadic appearance of recessives in supposedly pure lines is really due to mutation. The imaginary influence of past hybridization is a bogey that need not bother us.

Although recessive mutations and recombinations following hybridization may contribute much to specific and varietal diversity, it is obvious that they cannot bring about the origin of any fundamentally new type. We cannot, therefore, have any comprehensive knowledge of the genetic relationships of organisms until we know something about progressive mutation. Mendelian researches have shown us how to verify supposed relationships in the simplest cases, those in which characters have been lost. But can we always be sure that the recessive is the derived form? Is it not sometimes true that the dominant is the derived form, and that a new character has appeared? On this point the evidence is very unsatisfactory. Cases of progressive evolution have indeed been observed, but for the most part they have occurred out of the beaten track of the Mendelian and have been largely discounted, for Mendelism is the present fashion in genetics. In *Primula sinensis*, however, Professor Keeble has been fortunate enough to observe the origin of a Mendelian dominant.

There are two types of gigantism in Primula. In one type the chromosome number is the same as in the typical forms, and the giant acts as a simple Mendelian dominant when crossed with the parent. The other type is of a very different nature, and will be referred to later. A number of giants of the simple Mendelian type have been known among the cultivated varieties of Primula, but no one knew how they had originated until the "Giant White Queen Star" appeared as a mutation in the third guarded generation of a pure line of the "White Queen Star" variety. It came true from seed and has remained uniform through five successive generations, which included several hundred individuals. Although we cannot doubt in this case that a new dominant has arisen, there is unfortunately one flaw in the evidence. The new form has proved sterile when crossed with the parent form and with all other non-giant forms, so that its dominance is only inferred from its perfect analogy with other giants of the same type. The latter have been found by Gregory to show simple dominance over the non-giant forms. Our argument is based upon evidence fitted together from two sources. Other examples might be brought forward, but they are not as striking or as well authenticated as this one. There is a type of non-Mendelian progressive evolution, however, which has been far more satisfactorily studied. I refer to mutations in which new characters may be definitely associated with mutative changes in the chromosome number.

It appears that, in general, each species is characterized by a constant number of chromosomes. Within a genus, different species are frequently characterized by different chromosome numbers; and, exceptionally, within a species, well-characterized nominal varieties may differ in this respect. Lists of genera in which there is variation in chromosome number have been published by Gates and by Strassburger. Among the genera included in these lists, or concerning which information has been more recently published, we find the flowering plants Alchemilla, Antennaria, Crepis, Dahlia, Daphne, Drosera, Funckia, Hieracium, Humulus, Houttuynia, Musa, Oenothera, Primula, Rosa, Rumex, Spiranthes, Taraxacum, Thalictrum, and Wikstroemia, the ferns Athyrium, Lastrea and Nephrodium, the mosses Amblystegium, Bryum, Mnium and Phascum. The numbers range from 3 (x) and 6 ($2x$) in Canna to 48 (x) and 96 ($2x$) in Castalea. The processes of species formation must have been frequently attended by changes in the number of chromosomes. We cannot escape this conclusion when we take into consideration the two striking facts that within a species the number is constant, but that from species to species and from genus to genus it shows the greatest diversity.

Recent discoveries are making it very clear that mutative changes in the chromosome number occur frequently, and that such changes are always associated with a modification in the morphological characters of the plant. In other words, certain mutations are probably dependent upon, or, at any rate, closely associated with, visible changes in the nuclear mechanism. We have every reason to believe, therefore, that the different chromosome numbers of different species were acquired simultaneously with the acquisition of other specific characters.

Among the mutations of *Oenothera Lamarckiana* there is just one which de Vries regards as unquestionably progressive. It is the remarkable *Oenothera gigas*, which differs from its parent not only in numerous external characters but also in having twice as many chromosomes. The gametophytic and sporophytic numbers are 14 and 28 in *Oe. gigas*, 7 and 14 in *Oe. Lamarckiana*. Gates has shown that the double chromosome number of the former is correlated with a larger cell size in all corresponding tissues and that many of the gross characters of the plant are in turn dependent upon the difference in the cells. Hybrids between *Oe. gigas* and *Oe. Lamarckiana* show no semblance of Mendelian inheritance. In general they are very sterile, but in one case a fertile strain was obtained which remained constant through five generations. The hybrids are intermediate whichever way the cross is made, and, if back crossed with either parent, the secondary hybrids are again intermediate. Clearly we are not concerned here with a new dominant, in the Mendelian sense, but rather with the simultaneous origin of a whole group of non-Mendelizing characters, each of which is correlated with the increase in the number of chromosomes.

Among the flowering plants we do not know of any way in which the number of chromosomes may be experimentally modified. Among the mosses however, the brilliant work of the Belgian investigators Élie and Émile Marchal has shown that tetraploid races may be produced at will. Their results are of the greatest importance because of the light which they throw upon such spontaneous mutations as *Oenothera gigas*.

It has long been known that moss protonemata might be regenerated from bits of the seta or young capsule; in the past both Pringsheim and Correns have obtained such aposporous protonemata. The investigations of the Marchals were undertaken to determine (1) whether such protonemata would or would not give rise to moss plants with antheridia and archegonia, (2) whether, in case such plants were obtained, the gametes would have the $2x$ chromosome number of the normal sporophyte and give rise to a new sporophyte with the tetraploid chromosome number, and (3) whether the tetraploid races would be like the typical form of the species, or different. It was found that diploid gametophytes were obtained in which the vegetative cells were larger than in normal gametophytes and the generative cells were twice as large. The diploid gametophytes of dioecious mosses were absolutely sterile, and synoecious. They could be maintained in culture only by regeneration from pieces of the axis. In the case of the monoecious mosses the results were far more interesting, for the diploid gametophytes gave rise to tetraploid sporophytes, which produced good spores, and in turn reproduced the diploid gametophyte. The fertile races thus experimentally obtained were named *Amblystegium serpens bivalens* and *Amblystegium subtile bivalvens*. Cytological studies showed that the reproduction of these new races was by normal fusion of the diploid gametes. The cytological relations were worked out for Mnium, Bryum, Amblystegium and Phascum. In the case of Amblystegium it was found possible to double the chromosome number again by regeneration from the tetraploid sporophyte, but the tetraploid gametophytes thus obtained were completely sterile. The new bivalent race obtained from

Phascum was anomalous in that it was dwarf, rather than giant. It was sterile, but reproduced itself by propagula, which do not usually occur in the parent form, *Phascum cuspidatum*. Many mosses reproduce almost exclusively by vegetative means, and this experiment is in the highest degree suggestive of the manner in which this habit has come about. All of the other bivalent races differed from their parents in characters which obviously depended upon the greater size of the individual cells.

The experiments of the Marchals give us the strongest reason to believe that the visible differences between *Oenothera Lamarckiana* and *Oe. gigas* have been correctly interpreted as due to the doubling in the latter of the chromosome number. This explanation, first proposed by Gates, has been assented to by de Vries. The latter points out, however, that the genetical and physiological qualities of *Oe. gigas* are entirely different from those of *Oe. Lamarckiana*. The seeds of *Oe. gigas*, for example, are much more viable than those of the parent species, and germinate more quickly. *Oe. gigas* does not give the *laeta* and *velutina* twin hybrids when crossed with unrelated species, as *Oe. Lamarckiana* does. Obviously, then, the process of mutation has changed the hereditary qualities of the germ plasm even more than the morphological characters of the sporophyte. *Oe. gigas* gives rise to certain secondary derivatives which have no counterparts among the variations of *Oe. Lamarckiana*. Are we too optimistic if we view the former as a newly evolved center about which an entirely new series of specific variations may spring up?

It is natural to ask at this point how commonly mutative changes in the chromosome number occur. It is known that in *Oe. lata*, one of the most characteristic of the mutations from *Oe. Lamarckiana*, the unreduced number is 15 instead of 14. In *semigigas* mutations from *Oe. Lamarckiana* and *Oe. biennis* the number is 21. I have recently investigated the mutability of several species of Oenothera which belong to the small-flowered, self-pollinating series of forms which are generally lumped together under the name "*Oe. biennis*." Two of them have given rise to mutations characterized by an increased chromosome number. The cytological investigation of these new mutations has been undertaken by Mr. E. G. Arzberger, through whose kindness I am able to announce that *Oe. stenomeres* mut. *gigas* has 28 chromosomes, and that *Oe. pratincola* mut. *gigas* also has this number. The former seems in every way analogous to *Oe. gigas* de Vries, and will make it possible to determine the hereditary qualities of the latter much more satisfactorily than has thus far been possible. The method, of course, will be to compare the hybrids between the parent forms with those between the *gigas*-mutations. The crosses in both cases will be between gametes with the same number of chromosomes, so we need not expect the high degree of sterility which prevents the study of hybrids between the forms characterized respectively by 14 and 28 chromosomes. It would give increased significance to the discovery of the new *gigas*-mutations if tetraploid species of Oenothera should be found in nature. I mention this fact in the hope that everyone who is interested in the problem of mutation may be on the lookout for them. I already have one wild species in my garden which appears to possess certain traits of the *gigas*-forms but Mr. Arzberger was unable to get his preparations ready in time to make a chromosome count before this meeting took place.

It has already been mentioned that in the genus Primula two types of gigantism occur. One type is characterized by a doubling of the chromosome number and seems to represent the same type of mutation as that which in Oenothera gives rise to the *gigas*-form. Gregory has found that not only are the chromosomes doubled in the tetraploid Primulas, but also the Mendelian factors for numerous characters. The hereditary behavior of these mutations is, therefore, entirely different from that of the diploid races.

The differences in chromosome number which occur among species of the same genus represent changes which must necessarily have taken place abruptly. We can not imagine the origin of a *gigas*-race by gradual selection or by Mendelian segregation. Heribert-Nilsson has indeed offered a Mendelian explanation of *Oe. gigas*, but he has wholly neglected the cytological facts in the case. In *Oe. stenomeres* mut. *gigas* the chromosome count was made in the generation following the first appearance of the mutation. In the corresponding mutation from *Oe. pratincola* the count was made in the original mutation. There is the best of evidence, then, that the new chromosome number is acquired simultaneously with the new morphological characters. It is more reasonable to believe in a causal relationship between the cytological and morphological changes than to believe that the latter result from the hypothetical influence of hypothetical crossing in the indefinite past. It cannot be assumed that the modification of the chromosome number is itself due to Mendelian segregation, for there are too many facts which go to show that the chromosomes are themselves the mechanism by which Mendelian characters are transmitted. It is only necessary to call to mind Gregory's discovery that the Mendelian factors are doubled in tetraploid plants of Primula. If the mechanism is altered, the genetic qualities of the plant must of necessity be altered. For this reason I am disposed to lay especial emphasis upon those mutations in which a cytological change in the cell can be demonstrated, not, however, without stating my belief that mutations for which there is no visible basis are quite as independent of ancestral hybridization as the *gigas*-mutations. In certain groups, as the Coniferae for example, there is great uniformity in the chromosome number, showing that evolution has taken place through invisible modifications of the germ plasm. Probably most of the mutations which take place in any group of plants, even Œnothera, have the same chromosome number as the parent.

My *Oenothera* cultures of the last three years have given many mutations, aside from the *gigas* forms, which confirm in all essential points de Vries's experience with *Oe. Larmarckiana.* Some of them I regard as progressive, although it has not been possible yet to demonstrate that they are dominant in a Mendelian sense. In fact, Mendelian inheritance seems to play so small a part in Oenothera that in general we cannot expect to apply the test of dominance in judging of the progressiveness of mutations. We must consider a mutation as progressive when it shows characters which are not present in the parent.

It has been shown by de Vries that reciprocal hybrids between Oenothera species are frequently very unlike one another. Both de Vries and Davis have encountered cases in which the hybrids are strongly patroclinic. I have just the opposite experience with some of my interspecific hybrids, which are strikingly matroclinic. It is clear that in this genus reciprocal hybrids may be either matroclinic or patroclinic. In either case it is impossible to say that the characters of one parent or the other are dominant in the ordinary sense. With this explanation I shall proceed to a very brief discussion of two of my new mutations.

In *Oenothera pratincola* the seedling leaves are ovate. Seven different strains of this species have given rise to a mutation with round seedling leaves, which I have called mut. *nummularia.* In three strains the mutation has appeared in three successive generations, with a frequency of approximately one mutation for every 400 seeds sown. Correlated with the shape of the leaves are other characters, involving the size and branching of the plant and the pubescence and dehiscence of the calyx. The entire group of characters are coherent; it may be predicted from the shape of the seedling leaves alone that the other characters will appear in the mature plant. Nevertheless it is quite impossible to imagine any necessary inter-dependence between the characters which cohere in this mutation. No systematist who did not know the parentage of mut. *nummularia* could possibly decide which of two dozen elementary species in my garden had given rise to it.

Reciprocal hybrids between the parent species and the mutation appear to be strictly matroclinic, but the plants are still very young seedlings. The progeny of the cross mut. *nummularia* × *f. typica* are all mut. *nummularia,* conversely, the progeny of the reciprocal cross are all *f. typica*, except for the fact that mut. *nummularia* appears with its usual frequency of one plant in several hundred. It seems that only female gametes bear the group of characters which distinguish mut. *nummularia* from *f. typica.*

The other mutation which I wish to mention is *Oe. stenomeres* mut. *lasiopetala.* It differs from its parent species in a group of coherent characters, one of which is the hairiness of the petals. The solitary primary mutation when self-pollinated gave rise to a progeny consisting of typical *Oe. stenomeres* and mut. *lasiopetala* in a ratio suggesting 1 : 1, although the former was in excess. It is highly improbable that a Mendelian explanation will apply to this case, either, but I do wish to point out that such an explanation would necessitate viewing mut. *lasiopetala* as a dominant. A recessive could not have thrown the dominant parent.

As my experience with this highly interesting group of plants increases I am more and more convinced that de Vries' conception of the origin of species is the true one. He believes that new species, differing from the old ones in a coherent group of characters, may come into existance at one step, by mutation. The evidence for this special view of mutation has been doubted by several critics, who have brought forward several destructive arguments. I believe that all of these arguments can be met.

There is first the argument that *Oenothera Lamarckiana* is known only in horticulture, and may be a garden product; consequently that its behavior cannot be held to throw light upon the behavior of wild plants. It has now been shown that wild species show absolutely parallel phenomena.

It has also been said that the non-Mendelian behavior of Oenothera hybrids shows that the genus is so exceptional in its genetic behavior that it provides no basis for generalization. In reply we may say that the Mendelian school have in general confined their crosses to varieties of cultivated plants. If they had ventured into the field of interspecific hybridization they would have found plenty of parallels to the behavior of Oenothera. As a case in point we may cite the experience of W. Neilson Jones, who obtained matroclinic reciprocal hybrids between species of Digitalis. He did not think the results quite comparable to those of Oenothera for the reason that the Oenothera hybrids reported up to that time had been patroclinic. Now, however, this argument is removed by the discovery of matroclinic hybrids in Oenothera. The enormous literature of orchid hybridization contains frequent allusions to unlike reciprocal hybrids. In this largest family of the monocotyledons may be found numerous examples of both patroclinic and matroclinic hybrids. Many of them may prove to be cases of parthenogenesis, but the situation demands a much more thorough study than has yet been given it. The so-called "false hybrids" of Fragaria, as well as other Rosaceae, should be carefully investigated both cytologically and genetically. This much is sure; it is not yet time to speak of the universality of Mendelian phenomena, or of the exceptional nature of Oenothera. I am inclined to believe that such groups as the Orchidaceae may even provide parallels for the mutability of Oenothera. For example, Miss Pace has recently studied the cytology of *Spiranthes cernua* and *S. gracilis* from material collected near Chicago. Nine other species are interpolated between these two by Oakes Ames, in his Monograph of the American Species of Spiranthes. Yet they differ from one another in somewhat the same way that *Oenothera Lamarckiana* differs from *Oe. gigas*. Miss Pace finds 15 and 30 chromosomes in *Spiranthes gracilis*, as the reduced and unreduced numbers, but 30 and 60 in *S. cernua*. The latter may well be, as Miss Pace intimates, a tetraploid form of the former. Here is a fertile field for the experimentalist.

Jeffrey has lately argued that all plants which have any defective pollen grains are in a state of genetical impurity, and that any conclusions drawn from their genetical behavior, in connection with the vexed problem of the origin of species, must be subject to a large degree of reserve. Since all of the mutant Oenotheras are characterized by more or less defective pollen, he thinks that the mutations are segregates from hybrids, and that "the mutation theory of de Vries appears accordingly to lag useless on the biological stage, and may apparently be now relegated to the limbo of discarded hypotheses." Fortunately for the mutation theory, Professor Jeffrey's argument is not sound. In the first place it must be insisted that there can be no such thing as a morphological test of genetic impurity. We can only recognize genetical impurity by genetical tests. There is a certain sterile variety of the sweet pea, which, according to Jeffrey's pollen test, would be adjudged a hybrid. When crossed with forms with normal pollen it acts as a simple recessive, and like the other recessive varieties of this plant it has doubtless arisen by mutation. Bateson, who has critically studied this series of varieties, writes of, ". . . the sweet pea, a form which is beyond suspicion of having been crossed with anything else, and has certainly produced all the multitude of types which we now possess by variations from one wild species." Again, he states that "in spite of repeated trials, no one has yet succeeded in crossing the sweet pea with any other leguminous species." In the sweet pea, then, we find pollen conditions identical with those which Jeffrey believes are found only in hybrids; nevertheless there is no reason to believe that hybridization has ever occurred in the species. We may turn to another case. *Humulus Lupulus*, the hop, is normally dioecious, but monoecious individuals occur now and then which can hardly be considered as other than mutations. Winge has recently studied the pollen of one of these monoecious plants, found wild and transplanted from the woods into his garden at Carlsberg, Denmark. It bore staminate inflorescences at the base of otherwise hop-bearing branches. Cytological study showed that pollen mother cells were formed which divided normally but thereafter shrivelled up without making the tetrad division. Winge himself points out the similarity between this case and that of *Oenothera lata*. Of all the mutations from *Oenothera Lamarckiana*, *Oe. lata* is the most sterile. It is generally completely so, but two or three strains are now known which yield a small amount of good pollen. Monoecious hop plants likewise vary greatly in pollen fertility, and Winge has lately made pollinations with apparently quite normal pollen from a monoecious plant. Winge worked with the wild hop of northern Europe. Aside from geographic races of *Humulus Lupulus*, and the variations of it which have arisen in cultivation, there is no other form in the genus except *H. japonicus*. The former has 20 chromosomes in somatic cells, the latter 16. The cross *H. japonicus*×*H. Lupulus* cannot be made; the reciprocal cross yielded variously malformed embryos, none of which were capable of further growth. So in this case again there can be no suspicion that the pollen sterility has come about through hybridization.

All plants which show transition from hermaphroditism to dioecism or monoecism present conditions parallel to those in Humulus. The case of *Plantago lanceolata* happens to be especially familiar to me. In most strains of this species the flowers are all perfect and the pollen is good. There are other strains, however, which are gynodioecious. Half of the plants are normal hermaphrodites and the other half functionally pistillate. I say functionally pistillate because in many of these strains the anthers develop, and contain pollen, but the grains are much smaller than normal pollen and are not liberated by the dehiscence of the anthers. Probably they are non-functional. From this condition there are various gradations through strains in which the anthers of the functionally pistillate form contain no pollen to strains in which the stamens are replaced by staminodia. In the latter form the gynodioecious state is not only functionally but also structurally attained. *Plantago lanceolata* is an introduced weed in the United States. In the Old World the well-marked subgenus to which it belongs contains six other species, but all are of comparatively restricted distribution. In northern Europe, as in the United States, where the sex forms have been studied, there is no allied species with which it could hybridize. We have, therefore, no reason to suspect that anther sterility in *Plantago lanceolata* has any relationship to hybridization. On the contrary, we may assume that the dioecious states of the species have been attained by a series of mutations, and that pollen conditions simulating those in hybrids may come about by mutation as well as by hybridization.

At the risk of being tiresome there is one more type of anther sterility which I wish to touch upon. Bateson says: "Without much more critical data I suppose no one would nowadays be inclined to follow Darwin in instituting a comparison between the sterility of hybrids and that of illegitimately raised plants of heterostyle species. It is even difficult to imagine any essential resemblance between these two phenomena, nor has evidence ever been produced to show that illegitimately raised plants have bad pollen grains, which is the usual symptom of sterility in hybrid plants. . . ." This statement does not do justice to Darwin's evidence, which is quite convincing, especially in the case *Lythrum salicaria*. This species is trimorphic, *i. e.*, its flowers are long-styled, mid-styled or short-styled. Each flower type has two sets of stamens, coinciding in length with the styles of the two other types of flowers. Thus there are three kinds of stamens in the species and each bears pollen which is morphologically and physiologically different from that of the others. A pollination is legitimate when it takes place between a style and stamens of the same length. There are 18 possible combinations between the 3 forms, of which 6 are legitimate and 12 illegitimate. Darwin made all the different pollinations and found that only the legitimate ones were fully fertile. In regard to pollen sterility we will quote his own words. An illegitimate progeny from the long-styled form, pollinated from the longer or shorter stamens of the same form, consisted of 56 plants, belonging to three lots. "In several plants of all three lots, many of the anthers were either shrivelled or contained brown and tough, or pulpy matter, without any good pollen grains, and they never shed their contents; they were in the state designated by Gaertner as contabescent. . . . In one flower all the anthers were contabescent excepting two, which appeared to the naked eye sound; but under the microscope about two thirds of the pollen grains were seen to be small and shrivelled. In another plant, in which all the anthers appeared sound, many of the pollen-grains were shrivelled and of unequal sizes." An illegitimate progeny of nine plants resulted from the pollination of the short-styled form with pollen from the shorter stamens of the same form. "The anthers in many of the flowers on several plants were contabescent." Of 25 illegitimate plants from the mid-styled form, pollinated from the shorter stamens of the long-styled form, the pollen of 4 plants was examined "during the highly favorable season of 1866 . . . ; in one mid-styled plant, some of the anthers of the longer stamens were contabescent, but in the other anthers the pollen grains were mostly sound, as they were in all the anthers of the shorter stamens; in two other mid-styled and in one long-styled plant many of the pollen grains were small and shrivelled; and in the latter plant as many as a fifth or sixth appeared to be in this state." Darwin also expressly states that contabescent anthers occurred as a result of illegitimate pollination in *Primula sinensis* and *Primula veris*.

We can hardly fail to agree that Darwin was right when he said that defective pollen could arise within a single well-circumscribed species as a result of illegitimate pollination. We now know, thanks to the investigations of Bateson and Gregory, that heterostylism in Primula is inherited as a simple Mendelian character. Legitimate pollination maintains heterozygosis: illegitimate pollination, on the contrary, leads to the extraction of dominants which, in a sense, are genetically "purer" than the heterozygotes. Nevertheless, they may show, apparently as a result of their increased "purity," the very character of the pollen which Bateson and Jeffrey consider a sign of hybridity. A further interesting fact relating to Primula is as follows: strains of *P. sinensis* sometimes throw a mutation in which style and anthers are of the same length. This form, called equal-styled by Darwin, has been shown to be recessive to the long-styled form, which, in turn, is recessive to the short-styled form. Darwin cultivated several equal-styled races. He tells us that "my son, Mr. W. E. Darwin, . . . examined pollen from two equal-styled plants which he procured at Southampton; and in both the grains differed extremely in size, a large number being small and shrivelled, whilst many were fully as large as those of the short-styled form and rather more globular . . . The vast number of the small and shrivelled grains in the above two cases explains the fact that though equal styled plants are usually fertile in a high degree, yet some yield few seeds." Darwin tells us that his equal-styled races came true from seed, as, being extreme recessives, they must of course have done. Again we have a case of great genetic purity in association with defective pollen. There is no evidence, according to Bateson, that *Primula sinensis* has ever been hybridized. It seems to be one of the few cultivated plants in which great diversity has come about without any admixture with other species, although its purity is not as well attested as that of the sweet pea.

Examples might be multiplied indefinitely which show that defective pollen is as likely to indicate mutation as hybridization. In fact, I believe that it may be laid down as a rule that both processes are generally characterized by pollen sterility. With this conclusion in mind we may judge the mutation theory with a better chance of arriving at an unbiased decision.

Why is it that the polymorphic groups in which mutation is taking place, or supposed to be taking place, all show such undoubted evidence of the prevalence of hybridization? The answer is simple. The mutations, as a rule, are closely enough related to their parent species so that they hybridize readily with them. By hybridization the effect of a single mutation may be widely extended, for there is no experimental evidence that mutations can be "swamped out" by hybridization if otherwise fitted to survive. In groups which have perhaps long since passed their zenith and are now represented by a few very unlike species hybridization cannot readily take place because the species which remain are too unlike to hybridize. Mutation and hybridization are usually associated with one another, and I do not see how we can escape the conclusion that hybridization is subsidiary to mutation rather than mutation to hybridization. Both processes are simultaneously concerned in the evolution of such polymorphic genera as Oenothera, Rubus, Crataegus and Viola.

In the beginning of this paper three aims of the experimental geneticist were stated. The first, as we have seen, has been attained with fair success. The origin of many spontaneous variations has been observed, and their genetic and systematic characters have been studied. The second aim was to determine the causes of mutability, so as to be able to produce the condition at will. Mutations have indeed been produced experimentally in the case of the bivalent moss varieties which have already been referred to, but other work along this line has been unsuccessful. It is impossible to view as conclusive the experiments of MacDougal in which genetic variation is supposed to have been induced by the injection of various solutions into the ovaries of *Oenothera* (Raimannia) *odorata* and a species called "*Oenothera biennis*." Since MacDougal's views have had such wide publicity it may be well to summarize briefly the experimental evidence which he has brought forward. One mutation is said to have been induced in the strain called "*Oe. biennis*." This strain was started from presumably unguarded seeds of one individual mother plant, of which the first generation progeny were grown in 1904. We are nowhere given any idea how large this progeny was. Four plants were self-pollinated, and the progenies were grown from each in 1905. We are told that one progeny included 669 individuals, and that the rest were not counted. With this generation of 1905, the first to be grown from guarded seed, the injection experiments were made. An ovary was injected with 1 : 500 zinc sulphate solution. The seeds obtained gave a progeny, the size of which we are not told, in which the solitary mutation occurred. We are not told of any check cultures of sister plants from untreated ovaries, or of any further test of the strain for mutability. MacDougal referred to this experiment in 1911 as the most conclusive which he had yet performed, and stated that the mutation had come true from seed for five generations. There is no doubt that it is a very interesting mutation, especially in view of the fact that its behavior on crossing with the parent strain resembles that of *Oe. gigas*. The evidence that it was induced by the zinc sulphate solution is, however, quite insufficient. In the case of *Raimannia odorata* 13 mutations are said to have been obtained from ovaries which were injected in 1905. We are not told whether the strain had been previously purified by self-pollination or not. One ovary, injected with 10 per cent. sugar solution, gave two mutations; another, injected with 1 : 1,000 calcium nitrate solution, gave 10 mutations; a third, after exposure to radium, gave one mutation. We are not told whether the injected ovaries were all on one plant or not. There s no record of any check experiments with untreated ovaries, nor any record of the size of any of the cultures. More injections were made in 1906, with the contradictory result that calcium nitrate, which had been so potent the year before, induced no mutations at all. Three mutations were found in the progeny from "capsules" treated with 1 : 2,000 zinc sulphate, and also "other combinations" which were not followed in subsequent years. We are told, moreover, that the effect of the first injections with calcium nitrate persisted in following generations, for normal plants belonging to the strain from the ovary which was treated in 1905 with calcium nitrate gave rise to the same mutation which was supposed to have been induced by the chemical treatment. It seems to me that these facts can only mean that the strain used by MacDougal was in a highly mutable condition and that the experiments were not properly checked. It is especially noteworthy that Compton, also working with *Raimannia odorata*, has been unable to induce any mutations by the use of MacDougal's method. His strain was doubtless a non-mutable one. It is much to be hoped that more decisive results will attend future work along this line, and that whoever undertakes such experiments will adequately check them.

The third aim of the experimentalist was to provide a basis from which the systematist might determine deductively the genetic relationship of organisms. It must be admitted that except in the case of Mendelian varieties little progress has been made. Nevertheless certain negative conclusions which may be helpful may be made. In the first place, we must conclude that the degree of sterility which follows hybridization can not be used as an index of relationship. The swarm of 200 elementary species which are included under *Erophila verna* all differ from one another in relatively trivial characters. Rosen has shown that some of the hybrids among them are fully sterile and only one pair of the species which he tried gave fully fertile hybrids. Yet we can not doubt that all are closely related genetically. In the *Onagra* group of *Oenothera* there are many species which cross more readily with *Oenothera Lamarckiana* than the latter does with its mutation *Oe. gigas*. On the whole, we may say that among closely related forms neither interspecific sterility nor the lack of it is a true guide to the degree of relationship.

The origin of a form by hybridization should not be inferred from likenesses to both of the supposed parents, nor should a high degree of sterility be interpreted as a sign of hybridization. On the other hand, true interspecific hybrids in some cases show almost no influence of one or the other parent, and are as fertile as, or more fertile than, either parent.

The extreme morphological dissimilarity between some mutations and their parent species must teach us that little reliance can be placed upon the guesses of systematists regarding relationships in polymorphic groups. The herbarium botanist should fully realize the fact that his schemes of classification, in *Rubus* and *Crataegus* for example, are probably entirely artificial and do not represent natural relationships at all. Very important systematic characters may originate repeatedly and independently in unrelated lines of descent.

There is no test of what constitutes a species, except that it shall reproduce itself from generation to generation. Systematists should have a pragmatic attitude in describing species. Subdivision should extend as far as any one finds necessary. The geneticist needs to have definite designations for much smaller groups than the ecologist or morphologist is likely to be interested in. The makers of manuals should therefore endeavor to produce books which will supply the needs of either class of workers without misrepresenting facts. If it is necessary to have a more simple treatment of *Crataegus* than that of Sargent, for example, it may be done in such a way as not to discredit a large amount of careful work. The synonymy which is so conspicuous a feature of systematic work should be given a different significance.

There should be some discrimination between true synonyms, which are names applicable to the same identical organism, and the names of distinct units which it may not seem desirable to differentiate in a popular manual.

The problem of genetic relationships is the greatest problem of biology. Only repeated attacks, from every side, will solve it. It is no reason for pessimism that results come slowly. Centuries may pass before the greater trends of evolution will be understood. In the meantime we must not scorn our advances, even though they seem slight.

Bureau of Plant Industry,
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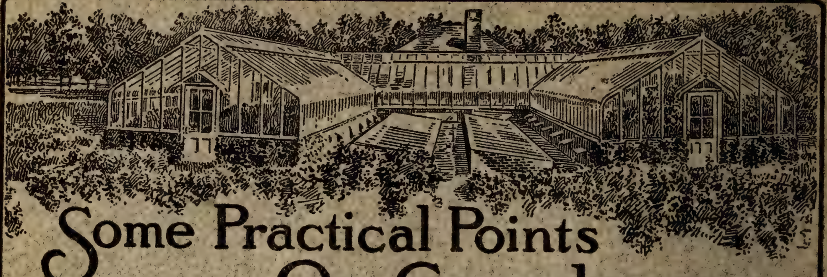

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A THREE-SALT NUTRIENT SOLUTION FOR PLANTS

JOHN W. SHIVE

From a survey of the literature bearing on the growth of plants in nutrient solutions, it appears that the experimental work most nearly approaching logical completeness is that recently carried out by Tottingham.[1] This writer employed 84 different solutions, all of approximately the same total osmotic concentration but each solution differing from all the others in its proportions of the four nutrient salts, mono-potassium phosphate, potassium nitrate, calcium nitrate, and magnesium sulphate. Besides these salts each solution contained the usual trace of iron, as ferric phosphate. Tests of the 84 different proportions of the nutrient salts, with total osmotic concentration (2.50 atmospheres diffusion tension) about the optimum for young wheat plants, showed that the best solution for the growth of tops (during the first four weeks after germination) contained the four salts in the following volume-molecular concentrations: KNO_3, .0049m.; KH_2PO_4, .0130m.; $Ca(NO_3)_2$, .0144m.; $MgSO_4$, .0145m. The solution giving the greatest dry weight of tops showed an improvement over Knop's solution, of the same osmotic concentration, of 11 percent on the basis of dry weight of tops grown in Knop's solution. The solution above described is Tottingham's best solution for tops, and may be regarded as the most reliable standard nutrient solution for wheat during its first four weeks of growth, so far worked out. The present writer has repeated the test of Tottingham's optimal series of solutions and of Knop's solution (all with osmotic concentration of 2.50 atmospheres) with wheat, and the results are in very satisfactory agreement with those obtained by Tottingham. For tops,

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[1] Tottingham, W. E. A Quantitative Chemical and Physiological Study of Nutrient Solutions for Plant Cultures. Physiol. Researches 1: 133–345. 1914.

[The **Journal** for March (2: 111–156) was issued 23 Ap 1915.]

the best growth (measured by dry weight) occurred with the same proportions of salts as those giving the best growth in the earlier work. While Tottingham reports an improvement of 11 percent over Knop's proportions, the corresponding improvement here shown was 12 percent. This agreement between the two results becomes still more pronounced when it is noted that the two experiments were carried out in different years and at different seasons.

Since the general problem of the salt requirements of plants largely remains to be studied, and since nutrient solutions will surely have to be employed in experiments bearing on this problem, it is quite essential that the standard solution used as a basis of comparison be as simple as possible and that it produce excellent growth of the plants. That distilled water is not suitable for use as such a standard is obvious from the fact: (1) that this medium removes salts from the plants, and (2) that the plants in pure water become visibly unhealthy after a few days and soon cease to grow at all. One phase of this matter has been dealt with by True and Bartlett[2] and by True.[3] It therefore seems highly desirable to devise a simpler solution than the four-salt mixture used by Knop and Tottingham, if this can be accomplished. An attempt was made in this direction, and the present announcement aims to state the main results in a preliminary way. A more complete account of this work will appear later.

Combinations of three nutrient salts, which contain all the essential elements required for plant growth besides iron, and which do not precipitate when mixed in solution of the needed concentration, are comparatively few. The solution here employed contained monopotassium phosphate, calcium nitrate, and magnesium sulphate; potassium nitrate is thus omitted from the Knop formula. These three salts contain all the essential elements except iron, and they dissociate in solution to form all of the ions present in the Knop-Tottingham four-salt solution. They do not readily precipitate when mixed in solution, and permit total concentrations suitable for plant growth. Thus the three-salt solution appears to be chemically adapted for the purpose in view. Growth tests are needed to show its suitability for supporting plant growth.

[2] True, R. H., and Bartlett, H. H. Absorption and Excretion of Salts by Roots, as Influenced by Concentration and Composition of Culture Solutions. U. S. Dep. Agr. Bur. Pl. Ind. Bull. 231. 1912.

[3] True, R. H. Harmful Action of Distilled Water. Amer. Journ. Bot. 1: 255–273. 1914.

A method similar to that used by Tottingham was here employed, to give a series of solutions differing in the proportions of the three salts, but all having approximately the same total osmotic concentration as measured by the lowering of the freezing point.[4] A trace of ferric phosphate was added to each solution. Each series contained 36 different solutions, instead of the 84 necessitated by the four-salt solution used by Tottingham. The plants here used were wheat (*Triticum vulgare*) (from the same lot of seed as was used by Tottingham) and buckwheat (*Fagopyrum esculentum* Moench.), and the seedlings were germinated and mounted in a manner similar to that employed by the earlier writer. All the three-salt solutions used in this comparison, including that with Tottingham's best proportions for wheat tops and the one with the regular Knop proportions, had approximately the same total osmotic concentration, but this was much lower than the concentration employed by Tottingham. They had a diffusion tension of 1.75 atmospheres (as measured by the lowering of the freezing point), while Tottingham's corresponding series showed 2.50 atmospheres. Both these concentrations lie within the optimum range for wheat. Tottingham's best solution for tops and the regular Knop's solution were included in this series for purposes of comparison. The cultures lasted 24 days, with change of solution every three days, the containers holding 250 cc.

The solution giving the best growth of wheat tops contained the three salts in the following volume-molecular partial concentrations: KH_2PO_4, .0180m.; $Ca(NO_3)_2$, .0052m.; $MgSO_4$, .0150m. This showed an improvement over Knop's solution, of the same total osmotic concentration, of 27 percent, while Tottingham's best solution here showed a corresponding improvement of but 16 percent. In the solution yielding the greatest dry weight of buckwheat tops the volume-molecular partial concentrations of the three salts were: KH_2PO_4, .0144m.; $Ca(NO_3)_2$, .0052m.; $MgSO_4$, .0200m. The improvement produced by this medium, in dry weight of buckwheat tops, over Knop's solution of the same total osmotic concentration, was 61 percent. The increase shown by Tottingham's best solution was 21 percent. The data here given represent averages from two series, one conducted in August and the other in October. The two corresponding series agreed, both for wheat and for buckwheat, in the proportions of the three salts giving the greatest dry weight of tops. These results are expressed in the tabulated form below, with those of Tottingham's tests added for comparison.

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[4] Shive, J. W. The Freezing Points of Tottingham's Nutrient Solutions. Pl. World 17: 345–353. 1915.

RELATIVE DRY WEIGHTS OF TOPS OF WHEAT AND BUCKWHEAT GROWN IN VARIOUS SOLUTIONS

Experimenter and Plant	Four-salt Solution				Three-salt Solution	
	Knop's		Tottingham's Best for Wheat Tops		Shive's Best (1.75 Atm.)	
	(2.50 Atm.)	(1.75 Atm.)	(2.50 Atm.)	(1.75 Atm.)	For Wheat Tops	For Buckwheat Tops
Tottingham (wheat)	1.00	——	1.11	——	——	——
Shive (wheat)	1.00	——	1.12	——	——	——
Shive (wheat)	——	1.00	——	1.16	1.27	——
Shive (buckwheat)	——	1.00	——	1.21	——	1.61

From these results it is clear that the three-salt mixture, with proper proportions, is not only eminently suitable for plant growth but that it gives a markedly better growth of tops than does either Tottingham's or Knop's four-salt solution, at least with a total osmotic concentration of 1.75 atmospheres, which is a suitable concentration for general water-culture work.

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PRELIMINARY NOTE ON THE MORPHOLOGY OF GNETUM

W. P. Thompson

The writer has collected and examined abundant embryological material of several species of Gnetum in the hope of securing information concerning the affinities of the Gnetales, and of throwing light on the origin of the gametophytic conditions and endosperm of the Angiosperms. Some of the results of the study are enumerated below. The detailed information will be published shortly.

1. The pollen grains sometimes germinate well up in the so-called "style," the pollen tubes then growing down to the nucellus as in the Angiosperms. Usually, however, germination takes place on the tip of the nucellus.

2. In the male gametophyte no prothallial cells are produced. Distinct male cells are formed.

3. *Only free nuclei* (*never cells*) are formed in the embryo-sac before the pollen tube enters, though cells are formed before fertilization takes place.

4. *One or more eggs are definitely organized* just before the pollen tube enters, usually while it is pressed against the side of the sac.

5. Before fertilization takes place the female gametophyte becomes divided into a large number of multinucleate compartments. All the nuclei in each compartment then *unite to form a fusion nucleus*. Fertilization is delayed until the fusion nuclei are formed.

6. The endosperm is formed by the divisions of the fusion nuclei in the lowermost compartments.

7. Coenocytic pro-embryonal tubes are formed which grow down into the endosperm and branch freely.

The bearing of conclusions five and six on the morphology of the endosperm of Angiosperms is obvious. The whole study indicates a close relationship between Gnetum and the Angiosperms.

University of Saskatchewan.

THE PERSISTENCE OF VIABLE PYCNOSPORES OF THE CHESTNUT BLIGHT FUNGUS ON NORMAL BARK BELOW LESIONS

R. A. Studhalter and F. D. Heald

Introduction

A study of the part taken by birds and insects in the dissemination of the chestnut blight fungus, *Endothia parasitica* (Murr.) And., has shown that both birds (6, 7) and insects (9, 10) can carry high numbers of pycnospores of this fungus. There are a number of possible sources from which these pycnospores may have been obtained, but it was thought that by far the greater number of them were brushed from both diseased and healthy chestnut bark during the normal movements of the birds and insects over these surfaces. This supposition was based primarily upon the fact, previously reported (4, 5), that pycnospores of the chestnut blight fungus, generally called the summer spores, are produced in large numbers even during the winter months, when only an occasional spore-horn can be found, and that they are washed down the trunks of trees with every rain.

It is natural to suppose that a large number of the pycnospores thus carried down the tree trunks would lodge on healthy bark below lesions. The work herein reported was undertaken in order to obtain some definite information on the numbers of pycnospores which find lodgment on normal bark, and the length of time they may remain viable after a rain.

A preliminary test of seven pieces of normal chestnut bark was made by Mr. M. W. Gardner in February, 1913, in which he obtained from 130 to 4,700 viable pycnospores per square centimeter of bark surface on the day following a rain.

Method

Pieces of smooth chestnut bark about 4 × 4 cm. in area were cut out with a flamed scalpel at varying distances below blight lesions. Without touching the bark it was dropped into an envelope which had previously been folded crosswise, placed in another envelope and sterilized. By this means the bark was transported to the laboratory where the tests were made on the following day.

In the laboratory 100 cc. of sterile tap water was poured into a shallow sterile dish. The piece of bark to be tested was trimmed down to 2 × 3 cm., stood on end in the dish of water, held by the corners and the outer surface scraped thoroughly with a cutting needle. The scalpels and needles were flamed before using, the hands and arms washed in mercuric chloride, and the entire operation carried on in a culture room. The dish was shaken at intervals for several hours, after which time 1 cc. of its contents was transferred to a flask containing 100 cc. of sterile tap water. This flask was also permitted to stand for at least an hour, and was shaken at intervals. From this flask 1 cc. and fractions were transferred to sterile Petri dishes; and it was also found advantageous in many instances to pour several plates using fractions of 1 cc. from the wash water. Chestnut bark agar was used in all the cultures.

Incubation took place at laboratory temperatures and the plates were carefully watched for at least ten days. This was necessary on account of the fact that many of the spores of the blight fungus, although viable, would not begin to germinate at once, and colonies of *Endothia parasitica* frequently made their appearance 3 to 5 days after they were due under normal conditions.

Discussion of Results

Six series of tests, representing 36 pieces of healthy chestnut bark, were made between December 22, 1913, and June 4, 1914. Of these 36 pieces only five failed to yield positive results. The remaining 31 showed the presence of viable pycnospores of the chestnut blight fungus in numbers varying from 33 to 172,222 per square centimeter of bark surface (Table I). None of the colonies appeared in cultures at the time at which ascospore colonies usually show (3), but it should be stated that it was impossible to tell from the time of appearance of the colonies that all originated from pycnospores, on account of the frequency of delayed germination. It is a known fact, however, that the ascospores are forcibly ejected into the air, and that none are washed down the tree trunks as is the case with pycnospores (4, 5). We therefore feel certain that the colonies appearing in the cultures originated from pycnospores.

Positive results were obtained almost uniformly during December

TABLE I

Results Obtained from the Tests of Healthy Chestnut Bark Below Blight Lesions

Series 1

Collected at Martic Forge, Pa., in afternoon of 12/22/13.

Rainfall night of 12/21/13, 0.13 inch.

Date of cultures, 12/23/13.

Series 2

Collected at Martic Forge in afternoon of 1/5/14.

Rainfall 1/3–4/14 and snow (melted), 1/4–5/14, 2.11 inches.

Date of cultures, 1/6/14.

Number of Test	Distance Below Blight Lesion	No. of Viable Pycnospores of *E. parasitica* per Sq. Cm.	No. of Viable Spores of Other Fungi per Sq. Cm.	Number of Test	Distance Below Blight Lesion	No. of Viable Pycnospores of *E. parasitica* per Sq. Cm.	No. of Viable Spores of Other Fungi per Sq. Cm.
1	30 cm.	15,556	1,111	8	25 cm.	3,889	1,111
2	70 "	21,667	2,778	9	65 "	566	0
3	43 "	6,111	411	10	38 "	0	4,167
4	37 "	4,688	7,813	11	32 "	3,333	2,778
5	57 "	6,771	2,604	12	45 "	28,125	2,604
6	39 "	833	833	13	33 "	2,500	6,667
7	34 "	12,778	23,333	14	30 "	24,242	10,101

Series 3

Collected at Martic Forge in afternoon of 1/26/14.

Rainfall, 1/24/14, 1.21 inches.

Date of cultures, 1/27/14.

Series 4

Collected at West Chester, Pa., in afternoon of 1/26/14.

Rainfall, 1/24/14, 1.35 inches.

Date of cultures, 1/27/14.

15	2 cm.	4,611	1,854	22	10 cm.	633	649
16	2 "	667	1,222	23	10 "	256	51
17	1 "	708	0	24	15 "	1,975	814
18	28 "	3,764	3,667				
19	2 "	6,500	5,729				
20	1 "	1,458	5,302				
21	1 "	1,431	7,500				

Series 5

Collected at West Chester, in afternoon of 5/26/14.

Rainfall, 5/12/14, 0.56 inches.

Date of cultures, 5/27/14.

Series 6

Collected at West Chester, in afternoon of 6/5/14.

Rainfall, 6/4/14, 0.30 inches.

Date of cultures, 6/6/14.

25	0.5 cm.	0	0	34	1 cm.	17,593	0
26	1 "	33	167	35	at edge	35,185	0
27	1 "	139	641	36	2 cm.	172,222	5,556
28	0.5 "	67	33				
29	5 "	67	100				
30	1.2 "	0	0				
31	.6 "	1,181	694				
32	5 "	0	0				
33	at edge	0	417				

TABLE II

Summary of Results Obtained from the Tests of Healthy Chestnut Bark below Lesions

Series	Place of Collection, Pa.	Last Rain Before Test		No. of Days Between Rain and Time of Collection	No. of Tests Made	No. of Viable Pycnospores per Sq. Cm. of Bark		
		Date	Am't., in.			Max.	Min.	Average
1	Martic Forge	12/21/13	0.13	1	7	21,667	833	9,772
2	" "	1/3-5/14	2.11	0	7	28,125	0	8,951
3	" "	1/24/14	1.21	2	7	6,500	667	2,734
4	West Chester	1/24/14	1.35	2	3	1,975	256	955
5	" "	5/12/14	0.56	14	9	1,181	0	165
6	" "	6/4/14	0.30	1	3	172,222	17,593	75,000

and January. Snow was on the ground during one collection, and not a single spore horn was found during these two months. That many pycnospores washed down by the winter rains (4, 5) remain clinging to healthy bark below lesions is definitely shown by the first four series in Table I, and the averages in Table II.

It is to be expected, of course, that much higher results would be obtained during the summer, when pycnospores are formed in much larger numbers and spore-horns are plentiful. The single series tested in June, collected on the day following a rain of 0.30 of an inch yielded from 17,593 to 172,222 viable spores per square centimeter. The average for the June series was 75,000 per square centimeter (483,900 per square inch), while the average for the four series in December and January was 6,378 per square centimeter (41,151 per square inch).

The distances below lesions at which the pieces of bark were taken varied from 0 to 70 cm., several pieces being cut at the very edge of the lesion. It is impossible to make any definite comparison between the number of viable spores obtained at the edge of a lesion and from points further down, for no two pieces of bark were cut below the same lesion at the same time, and no two lesions can readily be compared. From the data on hand, however, it would appear that the distance below the lesion has very little influence on the number of viable pycnospores present. Since pycnospores were very plentiful at the maximum distance tested, it seems certain that positive results could have been obtained at much greater distances.

The majority of tests showed that spores of fungi other than *Endothia parasitica* were also present on the bark (Table I). It is interesting to note, however, that in the majority of the cultures a larger number of colonies of *Endothia parasitica* appeared than of all other fungi combined, and that all of the plates from four pieces of bark developed only pure cultures of the chestnut blight fungus.

Relation to Rainfall

With the exception of Series 5, all of the collections were made not more than two days after a rain. Series 5 was collected at West Chester on May 26. The last preceding rain, 0.56 in., fell on May 12. During this interval of 14 days the weather was generally fair and warm, offering very good opportunities for the desiccation of the spores. Of the nine pieces of bark tested in this series, five yielded viable pycnospores, ranging in number from 33 to 1181 per square centimeter (Table I). Assuming that the average number of pycnospores on the day following the rain was about 75,000 per square centimeter, as was the case in Series 6 (Table II), the number which remained viable at the end of 14 days of desiccation was only a fraction of one percent of those present at the start. The significant fact, however, is that some pycnospores could withstand two weeks of desiccation in five out of nine pieces of bark tested.

After some rains the bark remains wet or moist in sheltered places for several days. Some of the bark tested was still wet at the time of collection. This was true for all of Series 2 and part of Series 1. On such areas of bark practically all of the pycnospores present would no doubt remain viable until the bark begins to dry. When the bark once begins to dry the number of viable pycnospores is probably greatly reduced by the mere act of drying, after which the further reduction will be more gradual. This statement is based upon the results obtained under certain artificial conditions (8) where the mere act of drying was found to reduce very greatly the number of viable pycnospores; once dry, the decrease was quite gradual.

It is brought out in the last mentioned work (8) that the decrease in the percentage of viable pycnospores is not as great when only a part of the mucilaginous coating is washed off, as when all the mucilage has been removed. Under natural conditions in the field it is very probable that only a small part of the mucilage is washed away by rains, for it may frequently happen that fragments of spore-horns may remain intact with nearly all of the mucilage still surrounding the spores. This condition leads us to believe that the number of pycnospores to resist desiccation is greater on the bark below lesions than under the artificial conditions referred to, where practically no mucilage was left on the spores.

In this connection it might be stated that disease organisms have been found to resist desiccation on the seed of the host plant longer than on other material. Such is the case with the bacteria causing the black rot of cabbage (2). Spores of *Glomerella gossypii*, the cause of cotton anthracnose, retain their vitality much longer when dried on cotton seeds than on cover slips (1). Although little evidence is at hand to confirm the statement, it is not impossible that spores of *Endothia parasitica* will resist desiccation on chestnut bark much longer than on some other materials.

Practical Bearing of the Results on the Dissemination by Birds and Insects

The conclusion has been reached that birds, and especially migratory birds (6, 7), are capable of carrying large numbers of viable pycnospores of the blight fungus for considerable distances; and that insects (9, 10) may be important agents in the local dissemination of the blight. Birds were shown to be carrying pycnospores only; the same was true of nearly all of the insects from which positive results were obtained, although a very small number of ascospores were found on several beetles.

The question of the source of these pycnospores has already been touched upon in the introduction. The results herein reported bring out the following reasons in support of the belief that these pycnospores were obtained in the main, if not wholly, as a result of the brushing of birds and insects over diseased and normal chestnut bark:

1. Viable pycnospores are present in varying numbers on healthy bark below lesions after both summer and winter rains.

2. Large numbers of pycnospores can be obtained immediately after a rain, but they may be present in smaller numbers for at least two weeks.

3. The largest numbers of spores were invariably obtained from birds and insects from about two to four days after a rain, corresponding rather closely with the time at which the largest numbers of viable pycnospores are present on normal bark below lesions.

SUMMARY

Viable pycnospores of the chestnut blight fungus were found to be present on normal bark below lesions in numbers varying from 0 to 172,222 per square centimeter of bark surface (1,111,176 per square inch).

Of the 36 pieces of bark tested, only five failed to yield positive results, and four of these five were collected 14 days after a rain.

Viable pycnospores were obtained in all but one of 24 tests made during December and January, when no spore-horns were present in the field.

An abundance of viable pycnospores was obtained at as great a distance below a lesion as 70 cm., and it appears very likely that they could be obtained at much greater distances below cankers.

Most of the tests were made one or two days after a rain; in one series, however, tested 14 days after a rain of 0.56 inch, positive results were obtained from five of the nine pieces of bark from which cultures were made.

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A TAXONOMIC STUDY OF SETARIA ITALICA AND ITS IMMEDIATE ALLIES

F. Tracy Hubbard

This paper deals with those species of Setaria grouped by Ascherson and Graebner, Syn. Mitteleur. Fl. **2**^{1}: 74 (1899) under their super-species *Panicum viride* (that is *S. verticillata*, *S. viridis* including *S. ambigua* and *S. italica*) and joined by Kuntze, Rev. Gen. **2**: 767, 768 (1891) to form his *Chamaeraphis italica*. The study was undertaken with a view to determine what *S. italica* var. *germanica* really was, as material of various forms was passing under that name and it gradually involved a careful examination of the whole group,—especially of *S. viridis* and *S. italica* since the old line of separation of these species did not hold good. *S. verticillata* separated itself readily when confined to those specimens with retrorsely barbed setae.

Some recent authors have reduced *S. italica* to a variety or subspecies of *S. viridis*,—for example Ascherson and Graebner, Syn. Mitteleur. Fl. **2**^{1}: 77 (1899), as *Panicum viride B. P. italicum* and Briquet, Prodr. Fl. Corse **1**: 68 (1910), as *Setaria viridis* subsp. *italica*. This is readily understandable when we consider that length of panicle and size of spikelet, the key characters commonly employed to separate the two species, entirely fail to do so as these characters frequently overlap very strongly, though they hold good in the average specimens. Generally, the panicle of *S. viridis* is said to range up to 8.5 cm. in length while the length of spikelet is given as about 2 mm. Careful study of the material at hand with measurements of every specimen examined, shows a range of panicle dimensions from 1 to 15 cm. long by 4 to 14 mm. in diameter. The spikelets range from 1.8 to 2.7, commonly 2.2 to 2.5 mm. long. *S. italica*, on the other hand, is said to have a panicle from 10 to 20 cm. long and spikelets about 3 mm. long. My measurements show that in the denser, more spike-like forms,—grouped by Alefeld, Landw. Fl. 315 (1866), in his Sect. *Moharium* and best represented by subvar. *germanica* of this paper,—the panicle may be as short as 1 cm. and not more than 7 mm. in diameter while the spikelets of the whole species vary in length from 2 to 3.2, more commonly 2.6 to 3 mm.

In testing the stability of the various characters I found that ordinarily the second glume of *S. viridis* equaled the sterile lemma in length whereas in *S. italica* it was for the most part noticeably shorter. Furthermore the spikelets in *S. viridis*, as soon as they become at all mature, shell out the whole spikelet—quite readily—leaving a cup-like receptacle; whereas those of *S. italica* shell out the fruit[1] only leaving the first and second glume and sterile lemma behind. This appears to me to be a good specific character and the use of it as a key character in separating the specimens gave homogeneous groups.

The name *germanica* was first used by C. Bauhin, Theatr. Bot. 518, fig. (1658) as *Panicum Germanicum sive panicula minore.* Exactly what form of *S. italica* Bauhin had is impossible to state though he undoubtedly had one of the larger lobulate forms as he states that the panicle is nine inches rarely a foot long. It may be yellow, purple or black, but he fails to say anything about the length of the setae. The plate is rather indefinite, but represents panicles with short setae. While the name originated with Bauhin the first use of it after 1753 is *Panicum germanicum* Miller, Gard. Dict., ed. 8, no. 1 (1768). Miller describes the species as follows "Spica simplici cernua, setis brevioribus, pedunculo hirsuto," basing his name on C. Bauhin, Pinax 27 (1671). In the discussion of his species of *Panicum* he makes the following statements regarding *P. germanicum*, "The stalks are terminated by compact spikes, which are about the thickness of a man's finger at their base, growing taper toward their points, and are eight or nine inches long,[2] . . . ." The phrase "setis brevioribus" has undoubtedly led to the recent interpretation of *S. italica* var. *germanica* as a form with setae shorter than the spikelets, but does this phrase compare the length of the setae with the spikelets; why may it not be a term comparative with the following species, *Panicum italicum*, which according to Bauhin's plate in Theatr. Bot. 519 is a plant with long setae? I believe that this interpretation is correct as I have a photograph and spikelets of the Miller material in the British Museum which were kindly sent me by Mr. A. B. Rendle. This material was collected in Chelsea Garden in 1760 and is labeled "Panicum Germanicum sive panicula minore" and consists of a panicle and two leaves. As a centimeter scale has been placed on the sheet the measurements can be easily obtained. The leaves are respectively 30 cm. long by 14 mm. broad and 24 cm. long by 14 mm. broad; the panicle is dense, about 11.5 cm. long by 13 mm. in diameter, with the lobes scarcely noticeable except at the very base. The setae are quite visible in the photograph, decidedly exceeding the spikelets and the fragment sent me shows them to be green and more than twice the length of the spikelet. The fruit is quite immature, but unquestionably belongs in the straw-colored group. These characters enable me to identify the form as that named *Panicum italicum* var. *praecox* by Alefeld, Landw. Fl. 315 (1866) and taken up by Körnicke in Körn. & Wern. Handb. Getreideb. **1**: 276 (1885) which I have classed as subvar. *germanica*.

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[1] The term *fruit* is consistently used in this paper to mean the seed inclosed in the fertile lemma and palea.

[2] These measurements are too long for subvar. *germanica* and also do not agree with Miller's specimen.

Early in the study of the subject I realized that a second form of the species was causing some of the confusion as some authors referred back to *Panicum germanicum* Mill., others to *P. germanicum* Willd. Willdenow, Sp. Pl. **1**: 336 (1797), described a plant as *P. germanicum*, "P. spica composita coarctata, spiculis glomeratis, involucellis setaceis flore longioribus, rachi hirsuta." As will be noticed, at once, the setae are described as longer than the spikelets as opposed to the variety *β* "involucellis flore brevioribus." Also the fact that the spike is described as composite should be noted. Willdenow refers to Roth, Tent. Fl. Germ., but his description clashes with Roth's in the length of the setae. Through the courtesy of Prof. Engler, Dr. Pilger wrote me as follows about the Willdenow Herbarium specimen "Was im Herbarium Willdenow unter Panicum germanicum liegt ist eine kräftige Form von Setaria italica. Obere Halmblätter (die letzten unter der Rispe) 20 cm. und darüber lang, 15–17 mm. breit. Rispe 13 cm. lang, im oberen Teil sehr dicht, 1½ cm. in Durchmesser, an der Basis locker unterbrochen. Spelzen und Granen ± braunlich violett gefärbt. Granen ziemlich kurz. Deckspelze oval, glänzend, grünlich-gelb, fast garnicht gerunzelt, nur hier und da schwache Runzelung." At a later date Dr. Pilger sent me a few spikelets of the Willdenow material which have purplish setae noticeably longer than the spikelets, and have straw-colored fruit. These facts place *P. germanicum* Willd. in the form called *P. italicum* var. *macrochaetum* by Körnicke, in Körn. & Wern. Handb. Getreideb. **1**: 273 (1885) which I have renamed var. *Hostii* as Körnicke derived his name from *Pennisetum macrochaetum* Jacq. Eclog. Gram. **3**: t. 25 (1815–20), a species pictured and described as having green setae: *P. macrochaetum* Jacq. is referable to subvar. *germanica*.

Besides the two above mentioned there are other applications of the name germanica and a chronological table of some of these uses may be of interest.

(1768) Miller, Gard. Dict., ed. 8, no. 1 (1768) previously discussed.

(1788) Roth, Tent. Fl. Germ. **1**: 27 (1788); **2**: 71 (1789) as *Panicum germanicum.* A comparison with *P. italicum* is given in volume two and four points of difference noted: of these, "3 Spiculis conglobatis nec elongatis. 4 Involucellis floribus brevioribus: nec triplo longioribus." The exact form is indeterminable, one of the short-setaed variations of *Setaria italica.*

(1797) Willdenow, Sp. Pl. **1**: 336 (1797), previously discussed.

(1802) Koeler, Descr. Gram. 16 (1802) as *Panicum italicum* Var. 1 *Germanicum.* The main point of his description is "setis spiculas non superantibus." I cannot place this further than to say it is a form of the *Moharium* group with very short setae.

(1802) Host, Ic. Gram. Austr. **2**: 12, t. 15 (1802) as *Panicum germanicum.* The description covers the plate which is of a plant of the *Moharium* group—dense panicled—with rather long purple setae and straw-colored fruit and is readily determined as subvar. *Metzgeri.* The description is broad enough to include other forms.

(1805) Lamarck, in Lam. & DC. Fl. Fr. ed. 3, **3**: 14 (1805) as *Panicum italicum β Germanicum.* Lamarck says ". . . les fleurs sont entourées de barbes très-coutres dans le variété β, ..." The form is not determinable, but more stress is laid on the length of the setae than on the size or shape of panicle. Probably a form of the lobed group with short setae.

(1812) Beauvois, Agrost. 51, 169, 178 (1812) as *Setaria germanica.* Transfer of name, only, based on *Panicum germanicum* Willd. cf. p. 169. I prefer to leave the form unstated though as far as the name is concerned it would belong under var. *Hostii.*

(1817) Roemer and Schultes, Syst. Veg. **2**: 492 (1817) as *Setaria germanica.* The description is copied from Willd. Sp. Pl., but their remarks lead me to believe they had one of the other variations, which one I cannot decide.

(1827) Link, Hort. Berol. **1**: 219 (1827) as *Setaria germanica.* Part of the description reads "Setae sursum scabrae spiculis 6–8 plo longiores interdum brevissimae aut deficientes" showing that he had several variants.

(1829) Trinius, Spec. Gram. **2**: t. 199 (1829) as *Panicum italicum* var. *germanicum.* The plate shows a long, slender, cylindrical panicle with long setae. The form is indeterminable, but of the *Moharium* group or quite possibly a variation of *S. viridis* as suggested by the cup-like receptacles shown in one of the detail drawings.

(1829) Eaton, Man., ed. 5, 322 (1829) *as Pennisetum germanicum* based on *Panicum germanicum* Willd. What Eaton really had is difficult to say without seeing his specimen, but it certainly is not var. *Hostii* to which the Willdenow reference is referable as Eaton's description reads "spike compound compact: spikelets glomerate: rachis hirsute: bristles short."

(1838) Schrader, in Linnaea **12**: 430 (1838) as *Setaria italica β germanica.* Based on *Panicum germanicum* Roth, a form with short setae which I cannot place. The other references given are Host Gram. **2**: t. 17 [probably should be 15] which equals subvar. *Metzgeri* and Trin. Icon. t. [probably 199 is meant] which as before stated is indeterminable. There is no description given.

(1816–46) Baumgarten, Enum. Stirp. Magno. Transs. **3**: 277 (1816–46). References to the same page of this work are given for *Paspalum germanicum* and *Pennisetum germanicum.* I have not seen the work and do not know which one is correct or if both combinations are given nor have I any idea to what form the citation is referable.

(1847) Wood, Class-book Bot., ed. 2, 607 (1847) as *Setaria germanica.* There is nothing in the description to determine what form it applies to. The other editions of Wood are practically the same.

(1848) Parlatore, Fl. Ital. **1**: 114 (1848) as *Setaria germanica.* There is nothing to determine the form.

(1853) Ledebour, Fl. Ross. **4**: 471 (1853) as *Setaria italica β germanica.* All that can be gathered from the description and synonymy is that it treats of a form with the setae shorter than the spikelets.

(1877) Doell, in Mart. Fl. Bras. **2**^{2}: 165 (1877) as *Panicum italicum* var. *germanicum.* Based on *P. germanicum* Willd., but the other citations refer to other forms. The description commences "Setae longiores . . ." so that he certainly had a long-setaed form.

(1890) Richter, Pl. Eur. **1**: 28 (1890) as *Setaria itálica* b) *germanica.* Only a transfer of name based on *Panicum germanicum* Willd. and consequently referable to var. *Hostii.*

(1890) Beck, Fl. Nieder-Oesterr. **1**: 46 (1890) as *Setaria italica* *α) germanica.* Based on *Panicum germanicum* Willd. and hence referable to var. *Hostii* as to name and probably as to description. Exclude the synonym *P. italica* var. *brevisetum* Doell which is another form.

(1891) Kuntze, Rev. Gen. Pl. **2**: 767 (1891) as *Chamaeraphis italica δ germanica.* The synonymy given is *Panicum germanicum* L. non Roth; *P. maritimum* Lam. To what Kuntze refers as *P. germanicum* L. I am not certain as the following is the nearest approach to such a combination that I can find in Linnaeus' works and the only reference given, in Linnaeus Codex. Linnaeus, Mant. **2**: 323 (1771) "*italicum.* Panicum germanicum multo minus est, spica ovata, nec elongata." This is in the section of the Mantissa headed Observationes in Species Plantarum cum Emendationibus et Animadversionibus, and is certainly only a comment on the German form of *S. italica.* *Panicum maritimum* Lam. is indeterminable as to form.

(1897) Scribner, U. S. Div. Agrost. Bull. **6**: 32 (1897) as *Chaetochloa italica germanica.* Transfer, only, based on *Panicum germanicum* Mill. and consequently referable to subvar. *germanica.*

(1899) Ascherson and Graebner, Syn. Mitteleur. Fl. **2**^{1}: 77 (1899) as *Panicum viride* [subsp.] *B. P. italicum* B. *germanicum.* This refers to some form that has the setae a little longer than the spikelets.

(1900) Scribner and Merrill, U. S. Div. Agrost. Bull. **21**: 21 (1900) as *Chaetochloa italica germanica.* This is based on *Panicum germanicum* Mill. and is referable to subvar. *germanica* as to name and possibly to subvar. *Metzgeri* as to plant.

(1902) Hitchcock, in Bail. Cycl. Am. Hort. **4**: 1662 (1902) as *Setaria italica* var. *germanica.* Based on *Panicum germanicum* Mill., but the authority given for the combination is Richter who based his combination on *P. germanicum* Willd. which is a different form. Prof. Hitchcock describes the plant as follows: "A smaller form more nearly approaching the wild *S. viridis:* bristles much longer than the spikelets which would apply to subvar. *germanica* very well."

(1906) Dalla Torre and Sarntheim, Fl. Tirol. **6**^{1}: 158 (1906) as *Panicum italicum* stirps *germanica.* Based on *P. germanicum* Mill. and referable to subvar. *germanica* [exclude synonym *Setaria germanica* Beauv. which is uncertain as to form, cf. (1812) Beauvois].

(1908) Hitchcock, in Gray Man., ed. 7, 119 (1908) as *Setaria italica* var. *germanica.* For discussion see (1902) Hitchcock. The description and form referred to are not the same as in the Cyclopedia as

Prof. Hitchcock in the Manual describes the variety as follows, "GOLDEN-WONDER MILLET, which is more slender and has bristles shorter than the spikelets." This description would apply to several of the variations mentioned in this paper. The common name Golden Wonder Millet applies to forma *breviseta*, a very large, lobulate-panicled form.

KEY TO SPECIES TREATED.

a. Setae retrorsely barbed. *S. verticillata*
a. Setae antrorsely barbed. *b.*
  b. Spikelet articulate below the glumes; complete spikelet shells out leaving a cup-like receptacle. *S. viridis* and varieties
  b. Spikelet articulate above the glumes; fruit only shells out leaving the glumes and sterile lemma behind. *S. italica* and variations

SETARIA VERTICILLATA (L.) Beauv. Agrost. 51, 171, 178 (1812). I have not attempted to go into the involved synonymy of this species as it is readily distinguished from *S. viridis* var. *ambigua*,—which it otherwise resembles,—by the retrorsely barbed setae. From the material I have seen I am inclined to agree with Sir. J. D. Hooker, Fl. Brit. Ind. **7**: 80 (1896) in considering *S. Rottleri* Spreng. synonymous.

RANGE.—Sparingly introduced in Canada, in the United States, mainly eastwards, in Mexico and Central America. In Venezuela? and Brazil fide Doell. Common in Europe, occurring in several sections of Africa, in Asia Minor, British India, Philippines, Australia and Hawaii.

SETARIA VIRIDIS (L.) Beauv. Agrost. 51, 171, 178, t. 13, f. 3 (1812): R. & S. Syst. Veg. **2**: 488 (1817): Link, Hort. Berol. **1**: 218 (1827): Kunth, Rev. Gram. **1**: 46 (1829) [excl. syn. in part]: Reichb. Fl. Germ. Excurs. **1**: 29 (1830): Kunth, Enum. Pl. **1**: 151 (1833) [excl. β which refers to var. Weinmanni]: Reichb. Ic. Fl. Germ. **1**: 68, t. 47 [188] (1834); ed. 2, l. c. (1850): Koch, Syn. Fl. Germ., ed. 1, 773 (1837) [excl. portion refering to *S. Weinmanni*]: Parl. Fl. Ital. **1**: 111 (1848): Gray, Man., ed. 1, 615 (1848); Hitchc. in ed. 7, 118 (1908): Coss. & Dur. Expl. Sci. Alger. **2**: 36 (1854): Miq. Fl. Ind. Bat. **3**: 467 (1855–60): Griseb. Fl. Brit. W. Ind. 554 (1864): Lowe, Nat. Hist. Brit. Grasses 101, t. 31, f. B (1865): Schur, Enum. Pl. Transs. 723 (1866); ed. nov. l. c. (1885): Syme, in Sowerby, Engl. Bot., ed. 3, **11**: 13, t. 1693 (1873) [as to name, descr. and plate questionable]: Benth. Fl. Austral. **7**: 494 (1878): Boiss. Fl. Orient. **5**: 443 (1884): Hook. f. Fl.

Brit. Ind. **7**: 80 (1896): Wildem. & Dur. Prodr. Fl. Belg. **3**: 80 (1900), PRELINNAEAN REFERENCES: C. Bauhin, Theatr. Bot. 138, fig. (1658), *Gramen paniceum, sive panicum sylvestre simplici spica:* C. Bauhin, Pinax 8 (1671) *Gramen paniceum spica simplici . . .*: Scheuchzer, Agrost. 46 (1719) *Gramen paniceum, seu panicum sylvestre simplici spica* [the Bauhin reference only]. *Panicum glaucum β* L. Sp. Pl., ed. 1, **1**: 56 (1753). *P. viride* L. Syst. Nat., ed. 10, **2**: 870 (1759): Leers, Fl. Herborn. 13, t. 2, f. 2 (1775): Curt. Fl. Lond. fasc. **4**: no. 44, t. 262 [also cited as fasc. **4**: t. 5] (1782?): All. Fl. Pedem. **2**: 240 (1785): Vill. Hist. Pl. Dauph. **2**: 64 (1787): Roth, Teut. Fl. Germ. **1**: 27 (1788); **2**: 69 (1789): Lam. Tabl. Ill. **1**: 169 (1791): Hoffm. Deutschl. Fl. **1**: 21 (1791): Willd. Sp. Pl. **1**: 335 (1797): Lam. Encycl. **4**: 727 [err. typ. 737] (1798): Sm. in Engl. Bot. **13**: t. 875 (1801) [the plate seems to me to be *Setaria italica*]: Host, Ic. Gram. Austr. **2**: 12, t. 14 (1802): Koel. Descr. Gram. 10 (1802): Knapp, Gram. Brit. t. 10 (1804): Schrad. Fl. Germ. **1**: 240 (1806): Gaudin, Agrost. Helv. **1**: 17 (1811) [excl. syn. Host, Gram. Austr. **2**: t. 15]: Hornem. Hort. Hafn. 81 (1813): Eaton, Man., ed. 2, 339 (1818): Mert. & Koch, in Röhl. Deutschl. Fl., ed. 3, **1**: 469 (1823): Trin. Gram. Panic. 163 (1826): Gaudin, Fl. Helv. **1**: 152 (1828): Trin. Spec. Gram. **2**: t. 203 (1829): Bertol. Fl. Ital. **1**: 420 (1833) [excl. syn. *P. verticillatum* b *ambiguum* Guss.]: Doell, Fl. Grossherz. Bad. **1**: 233 (1857): Lehm. in Arch. Naturk. Livl., ser. 2, **11**: 137 [Fl. Poln.-Livl. 137] (1895): Dalla Torre & Sarnth. Fl. Tirol. **6**^{1}: 156 (1906). *P. laevigatum* Lam. Fl. Fr. **3**: 578 (1778). *P. crusgalli* O. F. Müll. non L. in Fl. Dan. **5** fasc. **15**: 3, t. 852 (1782) [as to plate, but not as to descr. or syn.]. *P. reclinatum* Vill. Hist. Pl. Dauph. **2**: 64 (1787) [this is considered by some authors to be a synonym of var. *Weinmanni*, but I can see no evidence to show that it really is the same]. *P. viride* var. *reclinata* (Vill.) Hoffm. Deutschl. Fl. **1**: 21 (1791). "*Panicum, quale Linnaei ?*" Krock. Fl. Siles. **1**: 88 (1793) [The date may be 1787 cf. Pritzel Thesaurus. It is questionable if Krocker intended to make more than a comment, but various authors have treated it as though it were a combination]. *P. bicolor* Moench, Meth. 206 (1794). *Pennisetum viride* R. Br. Prodr. **1**: 195 in obs. (1810) by implication only: combination first made by R. & S. Syst. Veg. **2**: 489 (1817) in syn.: Nutt. Gen. **1**: 55 (1818): Eaton, Man. ed. 4, 389 (1824): Torr. Fl. N. Y. **2**: 430 (1843). *Panicum viride* var. *majus* Gaudin, Agrost. Helv. **1**: 18 (1811) as "*β Panicum viride majus* N.": Gaudin, Fl. Helv. **1**: 152 (1828) as "*β maius*": Lehm. in Arch. Naturk. Livl., ser. 2, **11**: 137 [Fl. Poln.-Livl. 137] (1895) as "Var. b *major* Koch" [Koch did not make the combination]: Dalla Torre & Sarnth. Fl. Tirol. **6**^{1}: 157 (1906). *Setaria villosa* Beauv. Agrost. 51, 171, 178 (1812) fide Hook. f. Fl. Brit. Ind. *S. nana* Dum. Obs. Gram. Belg. 139 (1823) fide Hook. f. l. c. and Aschers. & Graebn. Syn. Mitteleur. Fl. *S. affinis* Schultes, Mant. **2**: 276 (1824) questionable synonym fide Hook. f. l. c. *Panicum viride* var. *humifusum* Lej. Rev. Fl. Spa 13 (1824) nomen: Lej. & Court. Comp. Fl. Belg. **1**: 52 (1828). *P. humile* Thunb. ex Trin. Gram. Panic. 164 (1826) in syn. sub. P. viride. *Setaria viridis* var. *nana* Dum. Florula Belg. 150 (1827): Wildem. & Dur. Prodr. Fl. Belg. **3**: 80 (1900). *Panicum viride* var. *nanum* (Dum.) Lej. & Court. Comp. Fl. Belg. **1**: 52 (1828). *P. viride* var. *longisetum* Doell, Rhein. Fl.128 (1843): Doell, Fl. Grossherz. Bad. **1**: 234 (1857). *Setaria penicillata* Nees ex Wall. Cat. n. 8640D (1848) nomen, fide Hook. f. Fl. Brit. Ind. *Panicum viridescens* Steud. Syn. Pl. Gram. 51 (1854) fide Miq. Fl. Ind. Bat. *P. muticum* Hort. Lips. ex Steud. l. c. 51 (1854) in syn. sub. P. viride. *Setaria viridis* var. *latifolia* Ambr. Fl. Tirol. Merid. **1**: 42 (1854). *S. panis* Jessen, Deutschl. Gräser 248, 249, in part, figs. 391, 392 (1863). *S. viridis* var. *nodiflora* Saccardo, in Atti Ist. Ven., ser. 3, **9**^{2}: 865 (1864). *S. arvensis* subsp. *viridis* (L.) Bruhin, in Bericht. Naturw. St. Gallen **1865–66**: 215 (1866): *S. arvensis* subsp. *viridis* var. *fallax* Bruhin, l. c. **1865–56**: 215 (1866). *S. chlorantha* Schur, Enum. Pl. Transs. 723 (1866); ed. nov., l. c. (1885) fide Aschers. & Graebn. Syn. Mitteleur. Fl. *Panicum panis* Jessen, in Meyer & Jess. Alberti Magni Veg. 523 in part (1867). *P. viride* var. *vulgare* Doell, in Mart. Fl. Bras. **2**^{2}: 173 in obs. (1877). *P. viride* var. *gigantea* Franch. & Sav. Enum. Pl. Jap. **2**: 162 (1879). *P. italicum* var. *viride* (L.) Körnicke, in Körn. & Wern. Handb. Getreideb. **1**: 272, 277 (1885). *Setaria viridis* var. *typica* Beck, Fl. Nieder-Oesterr. 46 (1890). *S. viridis* var. *typica* forma *communis* Beck, l. c. 46 (1890). *S. viridis* var. *typica* forma *major* (Gaud.) Beck, l. c. 46 (1890). ? *S. viridis* var. *secunda* Beck, l. c. 46 (1890). *Chamaeraphis italica* var. *viridis* (L.) Ktze. Rev. Gen. Pl. **2**: 767, 768 (1891). *C. viridis* (L.) Millsp. in Bull. W. Va. Agric. Exper. Sta. **2**: 466 [Fl. W. Va. 466] (1892): Porter, in Bull. Torr. Bot. Cl. **20**: 196 (1893): Beal, Grasses No. Am. **2**: 157 (1896). ? *S. viridis* var. *insularis* A. Terrac. in Ann. Ist. Bot. Roma **5**: 93 (1894): Dur. & Schinz, Consp. Fl. Afr. **5**: 775 (1894). *Ixophorus viridis* (L.) Nash, in Bull. Torr. Bot. Cl. **22**: 423 (1895). *Setaria* *viridis* var. *typica* Posp. Fl. Oesterr. Küstenl. **1**: 51 (1897) [an var. typica Beck?]. *S. viridis* var. *major* (Gaud.) Posp. l. c. **1**: 51 (1897). *Chaetochloa viridis* (L.) Scribn. U. S. Div. Agrost. Bull. **4**: 39 (1897): Scribn. & Merr. U. S. Div. Agrost. Bull. **21**: 19. f. 8 (1900): Nash, in Britt. Man., ed. 1, 90 (1901); ed. 3, 90 (1907). *Panicum viride* [subsp.] *A. P. eu-viride* Aschers. & Graebn. Syn. Mitteleur. Fl. **2**^{1}: 76 (1899). *P. viride* [subsp.] *A. P. eu-viride* A. I. **a**. **1**. *majus* (Gaud.) Aschers. & Graebn. l. c. **2**^{1}: 77 (1899). *P. viride* [subsp.] *A. P. eu-viride* A. I. **a**. **1**. a. *reclinatum* (Vill.) Aschers. & Graebn. l. c. **2**^{1}: 77 (1899). *P. viride* [subsp.] *A. P. eu-viride* A. I. **a**. **1**. b. *pygmaeum* Aschers. & Graebn. **2**^{1}: 77 (1899). *P. viride* [subsp.] *A. P. eu-viride* A. I. **b**. *nodiflorum* (Saccardo) Aschers. & Graebn. l. c. **2**^{1}: 77 (1899). *Setaria viridis* var. *humifusa* (Lej. & Court.) Wildem. & Dur. Prodr. Fl. Belg. **3**: 885 (1903). *Panicum viride* var. *fallax* (Bruhin) Dalla Torre & Sarnth. Fl. Tirol. **6**^{1}: 157 (1906). *Setaria viridis* subsp. *eu-viridis* (Aschers. & Graebn.) Briq. Prodr. Fl. Corse **1**: 68 (1910). *S. italica* subsp. *viridis* (L.) Thell. in Mem. Soc. Sci. Nat. Cherb. **38**: 85 (1911). *S. viridis* var. *australis* F. M. Bail. Compr. Cat. Queensl. Pl. 611 (1913).

An extremely variable species frequently approaching *S. italica* or *S. verticillata* in appearance. From the former it may be distinguished by the spikelets being articulate below the glumes and easily shelled out—entire—when nearing maturity, leaving a little cup at the end of the pedicel, whereas *S. italica* is articulate above the glumes and shells out the fruit only. Some few specimens show intermediate characters and point toward hybridity. *S. viridis* is considered by many to be the origin of *S. italica* which may well be the case, but to-day the two seem to be readily separated by the above-stated character. From *S. verticillata* it is at once distinguished by the antrorse barbs of the setae: *S. verticillata* has the setae retrorsely barbed.

There are many forms of the species which in most instances are not clearly differentiated and which pass into each other too thoroughly to seem worthy of nomenclatorial designation. Size of plant, size of panicle, color and length of setae are all variable, but not consistently enough so to separate along these lines. Seed color is usually the same, —when fully mature,—a dull straw-drab with darker mottlings, though immature it ranges from whitish-green through shades of green and yellowish-green to straw.

The plant is usually erect or sometimes slightly geniculate, simple or many branched at the base, 3 to 10 dm. tall [depauperate specimens sometimes 1 dm. or even less], the culms fairly stout. The leaves are commonly fairly broad and long acuminate, 5 to 30 cm. long, 4 to 15 mm. broad. The panicles vary in size and compactness sometimes being somewhat lobulate and interrupted toward the base, but not verticillately so, more or less blunt or tapering at the base and apex, 1 to 15 cm. long[3] [usually relatively thick when short], 4 to 14 mm. in diameter, the rhachis commonly pilose. The spikelets range in size from 1.8 to 2.7 mm. long—most commonly 2.2 to 2.5 mm.,—0.8 to 1.6 mm. broad. The second glume is commonly as long as the sterile lemma, but occasionally is slightly shorter. The setae are variable in length, but usually considerably longer than the spikelets and are ordinarily green or greenish-yellow, but are sometimes purple-tipped or even all dark purple. The fruit is slightly rugulose, generally more so than in *S. italica.*

RANGE.—Occurring pretty generally throughout the United States and Canada, usually near cultivated land. Found sparingly in Mexico and in Brazil—fide Doell. Common throughout Europe, no plants seen from Africa or Asia Minor, but in Asia it occurs in Russia, British India, China, Japan and Formosa.

SETARIA VIRIDIS (L.) Beauv. var. AMBIGUA (Guss.) Coss. in Coss. & Dur. Expl. Sci. Alger. **2**: 36 (1854): Boiss. Fl. Orient. **5**: 443 (1884): Beck, Fl. Nieder-Oesterr. 46 (1890) [excl. syn. *P. viride* var. *brevisetum* Doell]: Dur. & Schinz, Consp. Fl. Afr. **5**: 775 (1894): Halacsy, Consp. Fl. Graec. **3**: 333 (1904). *Panicum viride* L. misapplied by Desf. Fl. Atlant. **1**: 58 (1798) [excl. syn.] fide Guss. Fl. Sic. Syn. *P. verticillatum* b *ambiguum* Guss. Fl. Sic. Prodr. **1**: 80 (1827). *Setaria ambigua* Guss. Fl. Sic. Syn. **1**: 114 (1842): Lojac. Fl. Sic. **3**: 266 (1909). *S. verticillata* var. *ambigua* (Guss.) Parl. Fl. Palerm. **1**: 36 (1845): Parl. Fl. Ital. **1**: 111 (1848): Richter, Pl. Eur. **1**: 28 (1890): Brand, in Koch, Syn. Deutsch. Fl., ed. 3, **3**: 2689 (1905). *Panicum adhaerens* Subsp. 1 *P. verticillatum antrorsum* A. Br. in Ind. Sem. Hort. Berol. **1871**, App.: 7 (1871). ? *Setaria pseudo-verticillata* Hort. ex A. Br. l. c. **1871**, App.: 7 (1871) in syn. *S. glomerata* Hort. ex A. Br. l. c. **1871**, App.: 7 (1871) in syn. *Panicum ambiguum* (Guss.) Hausskn. in Oesterr. Bot. Zeitschr. **25**: 345 (1875): Dalla Torre & Sarnth. Fl.

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[3] These small panicles are the secondary ones, the primary panicles are rarely under 5 cm. long.

Tirol. **6**^{1}: 156 (1906). *P. viride* var. *subverticillatum* Doell, in Mart. Fl. Bras. **2**^{2}: 173, in obs. (1877). *Setaria decipiens* C. Schimp. ex Nym. Consp. 787 (1842) in syn. sub S. ambigua. *Chamaeraphis italica* var. *ambigua* (Guss.) Ktze. Rev. Gen. Pl. **2**: 768 (1891). *Panicum verticillatum* B. *ambiguum* (Guss.) Aschers. & Graebn. Syn. Mitteleur. Fl. **2**^{1}: 75 (1899). *Chaetochloa ambigua* (Guss.) Scribn. & Merr. U. S. Div. Agrost. Bull. **21**: 18, f. 7 (1900). *Setaria verticillata* subsp. *ambigua* (Guss.) Briq. Prodr. Fl. Corse **1**: 67 (1910). *Panicum verticillatum* var. *antrorsum* A. Br. ex Briq. l. c. **1**: 67 (1910) in syn.

Distinguished from the typical form of the species chiefly by the more open, verticillate or subverticillate panicle [especially toward the base] and by the main rhachis being scabrous or hispid on the angles instead of pilose. Setae antrorsely barbed otherwise in habit strongly suggestive of *S. verticillata.* The setae often not much exceeding the spikelets. Fruit commonly greenish.

This form is decidedly intermediate between *S. verticillata* and *S. viridis,* commonly resembling the former in its verticillate or subverticillate panicle [occasionally *S. verticillata* is compact and only distinguishable from *S. viridis* by the retrorse barbs of the setae and quite frequently *S. viridis* is somewhat subverticillate] and the latter in the direction of the barbs of the setae. Of the two characters, the second seems to me to be more stable and of greater specific weight than the form of the panicle which is extremely variable in *S. viridis,* in fact in most of the Setarias: consequently I consider this form better treated as a variety of *S. viridis* than as a variety of *S. verticillata.* Good botanists have treated it both ways and still others have considered it to be a separate species as will be noted in the preceding bibliography. I do not find any consistent characters to separate it specifically.

RANGE.—Reported as a ballast plant collected by Scribner at Camden, N. J. (cf. Scribn. & Merr. U. S. Div. Agrost. Bull. **21**: 18 (1900)) and specimens seen from Washington, D. C., and Mobile, Ala. The first a weed in the Grass Garden, the second a ballast plant. Frequent in Europe more especially southward and also known from Algeria in Africa.

SETARA VIRIDIS (L.) Beauv. var. WEINMANNI (R. & S.) Brand, in Koch, Syn. Deutsch. Fl., ed. 3, **3**: 2690 (1905): Fernald & Wiegand, in Rhodora **12**: 133 (1910). *S. Weinmanni* R. & S. Syst. Veg. **2**: 490 (1817). *Panicum purpurascens* Opiz, in Flora **5**: 266 (1822) nomen. *Setaria purpurascens* Opiz, Boehm. Gew. 12 (1823) fide Aschers. & Graebn. Syn. Mitteleur. Fl. ? *Panicum viride* var. *brevisetum* Doell,[4] Rhein. Fl. 128 (1843): Doell, Fl. Grossherz. Bad. **1**: 234 (1857): Aschers. Fl. Prov. Brandenb. **1**: 809 (1864). *Setaria arvensis* subsp. *viridis* var. *purpurascens* Bruhin, in Bericht. Naturw. Ges. St. Gallen **1865–66**: 216 (1866) nomen fide Dalla Torre & Sarnth. Fl. Tirol. ? *S. viridis* b *arenosa* Schur, Enum. Pl. Transs. 723 (1866); ed. nov., 723 (1885). *S. viridis* a *laevigata* Schur, l. c. 723 (1866); ed. nov., 723 (1885). *S. viridis* var. *fuscata* Harz. Landw. Samenk. **2**: 1260 (1885) fide Beck, Fl. Nieder-Oesterr. ? *Chamaeraphis italica* var. *viridis* forma *purpurascens* Ktze. Rev. Gen. Pl. **2**: 767 (1891). *Panicum viride* var. *minor* Koch, ex Lehm. in Arch. Naturk. Livl., ser. 2, **11**: 137 [Fl. Poln.-Livl. 137] (1895) [Koch did not make the combination]. ? *P. viride* [subsp.] *A. P. eu-viride* A. II. *brevisetum* (Doell) Aschers. & Graebn. Syn. Mitteleur. Fl. **2**^{1}: 77 (1899). *P. viride* [subsp.] *A. P. eu-viride* B. *Weinmannii* (R. & S.) Aschers. & Graebn. l. c. **2**^{1}: 77 (1899). ? *P. viride* [subsp.] *A. P. eu-viride* B. II. *arenosum* (Schur) Aschers. & Graebn. l. c. **2**^{1}: 77 (1899). *P. viride* var. *Weinmannii* (R. & S.) Dalla Torre & Sarnth. Fl. Tirol. **6**^{1}: 157 (1906). *P. Weinmanni* "R. & S." according to Dalla Torre & Sarnth. l. c. **6**^{1}: 157 (1906) in syn. [R. & S. placed the species under *Setaria*]. *Setaria viridis* var. *breviseta* (Doell) Hitchc. in Rhodora **8**: 210 (1906): Hitchc. in Gray Man., ed. 7, 119 (1908) [as to plants, possibly not as to name].

Distinguishable from the common form of *S. viridis* chiefly in its spreading habit [base usually geniculate], its narrower leaves, 2 to 6 mm. wide and its smaller more slender panicles 0.7 to 5 cm. long, 3 to 6 mm. broad. The culms are frequently purplish toward the base. The spikelets are commonly smaller than those of the species 2 to 2.3 mm. long with exceptions only 1.6 mm. and up to 2.4 mm. The setae are either purple or green and vary in length from about the length of the spikelet to about 6 mm. long.

The length of the setae is so variable (when longest only about 6 mm.) that it seems undesirable to separate var. *breviseta* (Doell) Hitchc. as the plants so named agree in every other respect except length of setae. Whether Doell's *Panicum* viride *β brevisetum* is the same I am not so certain as the only point brought out by Doell is the length of the setae which might equally well apply to the species proper.

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[4] I can find no evidence to prove conclusively whether this is a short-setaed variation of the species proper or of var. *Weinmanni;* usage places it with the latter.

For a further discussion of this variety cf. Fernald and Wiegand, in Rhodora **12**: 133 (1910).

Range.—Occurring in northeastern and central Canada, in the United States in New England and Illinois. Frequent in Europe and also found in Russia and in Arabia. Specimens from Alabama, New Mexico, Arizona, Mexico, one from Hungary and several from India seem intermediate between the variety and the species.

Other minor variations of *S. viridis* or perhaps better termed abnormalities are:

A forked form of which I have seen specimens from Missouri, from northern India and from Japan.

A viviparous form to which several names have been given. *Panicum viride* var. *viviparum* Bertol. Fl. Ital. **1**: 421 (1833) nomen: Doell, Rhein. Fl. 128 (1843): Lehm. in Arch. Naturk. Livl., ser. 2, **11**: 138 [Fl. Poln.-Livl. 138] (1895). *Setaria viridis* var. *vivipara* (Bertol.) Parl. Fl. Ital. **1**: 112 (1848) [as "b. *viviparum*"]: Lucas, in Correspondenzbl. Naturf. Ver. Riga, **12**: 185 [Repr. 27] (1862). *S. arvensis* subsp. *viridis* var. *vivipara* Bruhin, in Bericht. Naturw. Ges. St. Gallen, **1865–66**: 215, 216 (1866) nomen. *Panicum viride* monstr. *vivipara* (Bruhin) Dalla Torre & Saruth. Fl. Tirol. **6**^{1}: 157 (1906).

The only specimen I have seen is one from Dover, Maine, collected by *Fernald* in 1896 and this has only one viviparous panicle, the others being normal.

A few specimens show characters or combinations of characters intermediate between *S. viridis* and *S. italica* which point to hybridity: the specimens that I have seen showing this most clearly are: Rochester, New York; *Baxter*, no. 5—Garret County, Maryland; *Donnell Smith* in 1879 and Moscow, Idaho; *Henderson*, no. 2849 all of which are in the U. S. Nat. Herb.

The following combinations have been commonly referred as synonyms to *S. viridis*, but I believe that they are better referable to other species.

Panicum cynosuroides Scop. Fl. Carn., ed. 2, **1**: 50 (1772) [commonly referred to *S. viridis*, but I believe more correctly a synonym of *S. glauca:* cf. Roth, Tent. Fl. Germ. **2**: 70 (1789) sub P. glaucum].

P. geniculatum Lam. Encycl. **4**: 727 [err. typ. 737] (1798): Hornem. Cat. Hort. Hafn. 28 (1807 ?): Willd. Enum. Hort. Berol. 1031 (1809): Hornem. Hort. Hafn. 81 (1813): Doell, in Mart. Fl. Bras. **2**^{2}: 158 (1877) [probably equals *S. glauca* of which Lamarck says in the original description it is perhaps only a variety].

Setaria geniculata (Lam.) R. &. S. Syst. Veg. **2**: 491 (1817): Kunth, Enum. Pl. **1**: 150 (1833) [probably equals *S. glauca:* cf. *P. geniculatum* Lam.].

Pennisetum geniculatum (Lam.) Jacq. Eclog. Gram. **3**: 37, t. 26 (1815–20) [the plate is an excellent picture of *S. glauca*].

Panicum tejucense Nees, ex Trin. Gram. Panic. 162 (1826) in syn. sub P. glaucum: Nees, Agrost. Bras. 243 (1829) [equals *S. imberbis* cf. Doell, in Mart. Fl. Bras. 2^2: 157 (1877)].

P. dasyurum Willd. ex Nees, Agrost. Bras. 241 (1829) [probably equals *S. imberbis*].

Setaria tejucensis (Nees) Kunth, Enum. Pl. **1**: 150 (1833) [equals *S. imberbis:* based on *Panicum tejucense* Nees].

SETARIA ITALICA (L.) Beauv. [sensu amplissimo] Agrost. 51, 170, 178 (1812): R. & S. Syst. Veg. **2**: 493 (1817): Spreng. Syst. Veg. **1**: 305 (1825): Link, Hort. Berol. **1**: 220 (1827): Schultes, Mant. **3**, add. **1** ad Cl. III: 598 (1827): Kunth, Rev. Gram. **1**: 46 (1829): Reichb. Fl. Germ. Excurs. 29 (1830): Kunth, Enum. Pl. **1**: 153 (1833): Wood, Class-book Bot. 439 (1845); ed. 1860, 788 (1861): Gray, Man. 615 (1848); Hitchc. in ed. 7, 119 (1908): Parl. Fl. Ital. **1**: 113 (1848): Duthie & Fuller, Field & Gard. Crops N. W. Prov. & Oudh **2**: 5, t. 25 (1883) [yellow fruit]: Richter, Pl. Eur. **1**: 28 (1890): Hook. f. Fl. Brit. Ind. **7**: 78 (1896) [excl. syn. in part]: Hitchc. in Bail. Cycl. Am. Hort. **4**: 1662 (1902). PRELINNAEAN REFERENCES: Dodon. Stirp. Hist. Pempt. 4, Lib. 1, p. 498, fig. (1583) *Panici indici spica:* Lobel. Ic. Stirp. Pl. 42, fig. (1591) *Panicum aliud Indicum. Panicula vilosa:* Clusius, Rar. Pl. Hist. Lib. 6, p. CCXV (1601) *Panicum vulgare:* Parkins. Theatr. Bot. p. 1139 fig. & p. 1140 text (1640) *Panicum Indicum pannicula villosa:* C. Bauhin, Theatr. Bot. 518, fig. (1658) *Panicum Germanicum sive panicula minore* [mentions variation in color of fruit]: C. Bauhin, Theatr. Bot. 519, fig. (1658) *Panicum Italicum sive panicula maiore* [mentions variation in color of panicle and fruit]: C. Bauhin, Pinax 27 (1671) *Panicum Germanicum sive panicula minore* [mentions color variation in panicle and fruit]: C. Bauhin, Pinax 27 (1681) *Panicum Italicum sive panicula majore:* Morison, Hist. **3** Sect. 8, p. 188, t. 3, Panicum 1 (1715) *Panicum Germanicum sive panicula minore* [mentions color variation in fruit]: Morison, Hist. **3**, Sect. 8, p. 188, t. 3, Panicum 2 (1715) *Panicum Italicum sive panicula majore* [mentions color variation in friut]: L. Hort. Cliff. 26 (1737) *Panicum sativum:* Royen, Prodr. Hort. Lugd.-

Bat. 54 (1740) *Panicum spica composita aristis gluma brevioribus:* L. Hort. Ups. 19 (1748) *Panicum spica composita, aristis flosculo brevioribus:* Rumph. Herb. Amboin. **5**: 202, t. 75, f. 2 (1750) *Panicum indicum* [yellow fruit]. The plate shows a panicle with the tip digitate-divided]. *Panicum italicum* L. Sp. Pl. **1**: 56 (1753) [excl. syn. Gronov.]: Gouan, Hort. Monsp. 34 (1762): Mill. Gard. Dict., ed. 8, Panicum no. 2 (1768) [mentions different colors of fruit]: L. Mant. **2**: 323 (1771): Weig. Obs. Bot. 22 (1772): Willd. Sp. Pl. **1**: 336 (1797): Lam. Encycl. **4**: 728 [err. type 738] (1798) [mentions different colorings of setae]: Koel. Descr. Gram. 16 (1802): Host, Ic. Gram. Austr. **4**: 8 in part [excl. t.] (1809): Gaudin, Agrost. Helv. **1**: 20 (1811): Mert. and Koch, in Röhl. Deutschl. Fl., ed. 3, **1**: 470 (1823): Metzger, Eur. Cereal. 63 (1824) [as a whole]: Trin. Gram. Panic. 164 (1826): Doell, in Mart. Fl. Bras. **2**^{2}: 165 (1877): Lehm. in Arch. Naturk. Livl., ser. 2, **11**: 138 [Fl. Poln.-Livl. 138] (1895). *P. germanicum* Roth, Tent. Fl. Germ. **1**: 27 (1788); **2**: 71 (1789): Host, Ic. Gram. Austr. **2**: 12 in part [excl. t.] (1802). *P. glomeratum* Moench, Meth. 207 (1794). *P. elongatum* Salisb. Prodr. 18 (1796). *P. maritimum* Hort. Par. ex Lam. Encycl. **4**: 727 [err. typ. 737] (1798) [possibly belongs under subvar. *Metzgeri*, but I do not know the color of the fruit]. *P. italicum* var. *germinicum* Koel. Descr. Gram. 17 (1802): D.C. in Lam. & D.C. Fl. Fr., ed. 3, **3**: 14 (1805) [as *β P. germanicum*]. ? Trin. Spec. Gram. **2**: t. 199 (1829): Doell, in Mart. Fl. Bras. **2**^{2}: 165 (1877). *Pennisetum italicum* (L.) R. Br. Prodr. **1**: 195 (1810): Eaton, Man. Bot., ed. 3, 383 (1822); ed. 8, [Eaton & Wright, No. Am. Bot.] 346 (1840). *Setaria germanica* (Willd.) Beauv. Agrost. 51, 169, 178 (1812) [based on *P. germanicum* Willd.]: R. & S. Syst. Veg. **2**: 492 (1817): Link, Hort. Berol. **1**: 219 (1827): Reichb. Fl. Germ. Excurs. 29 (1830): Parl. Fl. Ital. **1**: 114 (1848). *Panicum intermedium* Vahl, ex Hornem. Hort. Hafn. **1**: 82 (1813) fide Trin. Gram. Panic 165 (1826): *P. compactum* Kit. ex Schultes, Oesterr. Fl., ed. 2, **1**: 212 (1814) in syn. sub P. germanicum var. *β*. *Echinochloa intermedia* (Vahl) R. & S. Syst. Veg. **2**: 477 (1817). *Setaria maritima* (Lam.) R. & S. l. c. **2**: 492 (1817). *Panicum sibiricum* Hort. ex R. & S. l. c. **2**: 493 (1817) in syn. sub S. italica *β*. *P. setosum* Hort. ex R. & S. l. c. **2**: 492 (1817) in syn. sub S. germanica *β* non Sw. *P. aegyptiacum* Hort. ex R. & S. l. c. **2**: 493 (1817) in syn. sub S. italica. *Pennisetum germanicum* Baumg. Enum. Stirp. Magno Transs. **3**: 277 (1816–46) fide Ind. Kew. **2**: 458 (1895). *Paspalum germanicum* Baumg. l. c. **3**: 277 (1816–46) fide Richter, Pl.

Eur. **1**: 28 (1890). *Panicum pumilum* Link, Enum. Hort. Berol. **1**: 76 (1821) fide Trin. Gram. Panic. 166 (1826) sub P. italicum f). *P. macrochaeton* Link, l. c. **1**: 76 (1821) fide Trin. l. c. 165 (1826) sub P. italicum d). *P. melfrugum* Hort. ex Mert. & Koch, in Röhl. Deutschl. Fl., ed. 3, **1**: 471 (1823) in syn. sub P. italicum. *P. asiaticum* Hort. ex Mert. & Koch, l. c. **1**: 471 (1823) in syn. sub P. italicum. *P. serotinum* Hort. ex Mert. & Koch, l. c. **1**: 471 (1823) in syn. sub P. italicum. *Setaria macrochaeta* (Link) Schultes, Mant. **2**: 274 (1824): Link, Hort. Berol. **1**: 218 (1827). *Panicum setaceum* Hort. ex Trin. Gram. Panic. 166 (1826) in syn. sub P. italicum f). *P. attenuatum* Hort ex Trin. l. c. 166 (1826) in syn. sub P. italicum f). *Pennisetum germanicum* (Willd.) Eaton, Man. Bot., ed. 5, 322 (1829); ed. 8, [Eaton & Wright, No. Am. Bot.] 346 (1840). *Setaria flavida* Horn. ex Reichb. Fl. Germ. Excurs. 29 (1830) in syn. sub S. germanica. *S. violacea* Horn. ex Reichb. l. c. 29 (1830) in syn. sub S. germanica. *S. asiatica* Hort. ex Reichb. l. c. 29 (1830) in syn. sub S. italica. *S. persica* Hort. ex Reichb. l. c. 29 (1830) in syn. sub S. italica. ? *S. globularis* Presl, Rel. Haenk. **1**: 314 (1830) fide Kunth, Enum. Pl. **1**: 152 (1833) and Hook. f. Fl. Brit. Ind. **7**: 78 (1896). *S. melinis* Link, Hort. Berol. **2**: 219 (1833). ? *Panicum miliaceum* Blanco, Fl. Filip., ed. 1, 39 (1837) fide Hook. f. l. c. **7**: 78 (1896). *Setaria italica β germanicum* (Roth) Schrad. in Linnaea **12**: 430 (1838). *Panicum vulgare* Wallr. in Linnaea **14**: 542 (1840) [based on P. vulgare Clus. Rar. Pl. Hist. Lib. 6, p. CCXV (1601)]. ? *P. globulare* (Presl) Steud. Nom., ed. 2, **2**: 257 (1841): Steud. Syn. Pl. Gram. 51 (1854). *Setaria italica β germanica* (Beauv.) Ledeb. Fl. Ross. **4**: 471 (1853). *S. japonica* Pynaert, in Fl. des Serres **14**: 7, fig. (1861). *S. panis* Jessen, Deutschl. Gräser 248, 249 ex parte (1863). *Panicum italicum* c) *maritimum* (Lam.) Aschers. Fl. Prov. Brandenb. **1**: 809 (1864). *Setaria compacta* Schur, Enum. Pl. Transs. 967 [index] (1866); ed. nov., 967 [index] (1885). *S. germanica* a. *legitima* Schur, l. c. 724 (1866); ed. nov., 724 (1885). *Panicum panis* Jessen, in Meyer & Jess. Alberti Magni Veg. 523 ex parte (1867). *P. italicum* var. *compactum* A. Br. Ind. Sem. Hort. Berol. **1871**: 4 (1871) nomen. *P. italicum* var. *japonicum* Hort. Ber. ex A. Br. l. c. **1871**: 4 (1871) nomen. *Setaria italica* var. *typica* Beck, Fl. Nieder-Oesterr. **1**: 46 (1890). *S. italica* var. *compacta* (Kit.) Beck, l. c. **1**: 46 (1890). *Chamaeraphis italica* (L.) Ktze. Rev. Gen. Pl. **2**: 767 (1891) in part: Beal, Grasses No. Am. **2**: 154 (1896). *C. italica α sativa* Ktze. l. c. **2**: 768 (1891). *C. italica β elobata* Ktze.

l. c. **2**: 768 (1891). *C. italica δ germanicum* Ktze. l. c. **2**: 768 (1891). *Ixophorus italicus* (L.) Nash, in Bull. Torr. Bot. Cl. **22**: 423 (1895): Nash, in Britt. & Br. Ill. Fl. **1**: 127, f. 283 (1896). *Chaetochloa italica* (L.) Scribn. U. S. Div. Agrost. Bull. **4**: 39 (1897): Scribn. & Merr. U. S. Div. Agrost. Bull. **21**: 20, f. 9 (1900): Nash, in Britt. Man. 90 (1901); ed. 3, 90 (1907): Nash, in Small, Fl. Southeast. U. S. 107 (1903). *Panicum viride* [subsp.] *B. P. italicum* (L.) Aschers. & Graebn. Syn. Mitteleur. Fl. **2**^{1}: 77 (1899). *P. viride* [subsp.] *B. P. italicum* B. *germanicum* Aschers. & Graebn. l. c. **2**^{1}: 78 (1899). *P. viride* [subsp.] *B. P. italicum* C. *maritimum* (Lam.) Aschers. & Graebn. l. c. **2**^{1}: 78 (1899). *Setaria italica* var. *germanica* "(Mill.) Richter," according to Hitchc. in Bail. Cycl. Am. Hort. **4**: 1662 (1902): according to Hitchc. in Gray, Man. ed. 7, 119 (1908) not var. *germanica* of Richter, Pl. Eur. **1**: 28 (1890) which is based on *Panicum germanicum* Willd. and equals var. *Hostii*. *S. viridis* subsp. *italica* (L.) Briq. Prodr. Fl. Corse **1**: 68 (1910).

NOTA.—The preceding references apply to some form of the species, but cannot be placed with certainty under any given variation.

S. italica is an exceedingly variable species which has been under cultivation for many centuries and consequently has developed innumerable strains. The size of the plant varies from well over a meter to only about a decimeter high and the thickness of the culm is equally variable. The leaves range in length from only about 3 cm. up to 50 cm. and in breadth from 5 to 30 mm.; commonly linear-lanceolate and long-acuminate, but occasionally relatively broad and short-acuminate. The panicle is more or less lobulate-compound and interrupted at the base [section *Maximum* of Alefeld, Landw. Fl.] to dense, seemingly spicate [section *Moharium* of Alefeld, l. c.] and ranges in length from 1 to 30 cm. or more and in diameter from 7 to 55 mm. The spikelets vary in size from 2 to 3.2 mm. long, more commonly 2.6 to 3 mm. and are always articulate above the glumes allowing the fruit to be easily shelled out. The second glume is usually slightly shorter than the sterile lemma, sometimes only three fourths as long but occasionally almost as long. The setae vary in color—being green, purple or brown—and in length from shorter than the spikelets to many times their length, but they are always upwardly barbed [sometimes very slightly so]. The fruit is of three main colors—yellowish, reddish or blackish,—but in varying shades. It is more or less rugulose, sometimes almost smooth when it is shiny.

Of the several lines of variation mentioned, seed-color seems to be the most stable; color of setae the next; form and size of panicle in the third degree, but very variable and intergrading; length of setae the fourth and extremely variable, but noticeable in the extremes; whereas size and shape of seed do not seem to offer any line of separation. Along these lines the following variants of the species may be noted, remembering always that distinctions based on form and size of panicle are sure to show numerous intergrades and that length of setae is only applicable in the extreme form.

KEY TO THE VARIANTS OF SETARIA ITALICA.[5]

a. Fruit yellowish to straw or light brown. *b.*	
b. Setae green. *c.*	
c. Panicle more or less open lobulate. *d.*	
d. Setae noticeably longer than spikelets.	subsp. *stramineofructa.*
d. Setae shorter than or barely exceeding spikelets.	forma *breviseta.*
c. Panicle dense or slightly lobulate at base. *e.*	
e. Setae noticeably longer than spikelets.	subvar. *germanica.*
e. Setae shorter than or barely exceeding spikelets.	forma *mitis.*
b. Setae purple. *f.*	
f. Panicle more or less open lobulate.	var. *Hostii.*
f. Panicle dense or slightly lobulate at base. *g.*	
g. Setae noticeably longer than spikelets.	subvar. *Metzgeri.*
g. Setae shorter than or barely exceeding spikelets.	forma *curtiseta.*
b. Setae brown. *h.*	
h. Panicle more or less open lobulate. *i.*	
i. Setae noticeably longer than spikelets.	var. *brunneoseta.*
i. Setae shorter than or barely exceeding spikelets.	forma *brachychaeta.*
h. Panicle dense or slightly lobulate at base.	subvar. *densior.*
a. Fruit reddish or orange. *j.*	
j. Setae green. *k.*	
k. Panicle more or less open lobulate. *l.*	
l. Setae noticeably longer than spikelets.	subsp. *rubrofructa.*
l. Setae shorter than or barely exceeding spikelets.	forma *gigas.*
k. Panicle dense or slightly lobulate at base.	subvar. *pabularis.*
j. Setae purple. *m.*	
m. Panicle more or less open lobulate.	var. *purpureoseta.*
m. Panicle dense or slightly lobulate at base.	subvar. *violacea.*
j. Setae brown. *n.*	

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[5] Attention is called to the fact that *a*, *b* and *j* each occur three times in the following key.

n. Panicle more or less open lobulate. *o.*
  o. Setae noticeably longer than spikelets. var. *rubra.*
  o. Setae shorter than or barely exceeding spikelets. forma *aurantiaca.*
n. Panicle dense or slightly interrupted at base. subvar. *condensa.*
a. Fruit blackish, brownish black or purplish black, with pale yellowish-straw fruits intermingled (these sometimes predominating). *p.*
  p. Setae green, panicle more or less lobulate. subsp. *nigrofructa.*
  p. Setae purple-brown, panicle dense. var. *atra.*

SETARIA ITALICA (L.) Beauv. subsp. **stramineofructa** nom. nov. *Panicum italicum* var. A Metzger, Eur. Cereal. 63, t. 19, f. A (1824). ? *P. italicum* Trin. Spec. Gram. 2: t. 198 (1829). *P. italicum* var. *longisetum* Doell, Rhein. Fl. 128 (1843): Doell, Fl. Grossherz. Bad. 1: 233 (1857): Körnicke, in Körn. & Wern. Handb. Getreideb. 1: 273 (1885): Wern. in Körn. & Wern. l. c. 2: 890 (1885). ? *P. italicum* var. *californicum* Körnicke, Syst. Übers. Cereal. 18 (1873): Körnicke, in Körn & Wern. l. c. 1: 273 (1885): Wern. in Körn. & Wern. l. c. 2: 891 (1885) [I have not been able to place this variety satisfactorily, I should judge it dealt with specimens intermediate between subsp. *stramineofructa* and subvar. *germanica*]. *P. italicum* var. *lobatum* Körnicke, in Körn. & Wern. l. c. 1: 273 (1885): Wern. in Körn. & Wern. l. c. 2: 890 (1885). *P. macrurum* Hort. ex Körnicke, in Körn. & Wern. l. c. 1: 273 (1885) in syn. sub P. italicum var. longisetum. *P. frumentaceum* Hort. ex Körnicke, in Körn. & Wern. l. c. 1: 273 (1885) in syn. sub P. italicum var. longisetum. *Setaria chrysantha* Hort. ex Körnicke, in Körn. & Wern. l. c. 1: 273 (1885) in syn. sub P. italicum var. longisetum. *P. viride* [subsp.] *B. P. italicum* A. *longisetum* (Doell) Aschers. & Graebn. Syn. Mitteleur. Fl. 2^1: 78 (1899).

Plant stout, 5 to 11 dm. or more tall, leaves linear-lanceolate, long tapering acuminate, 12 to 50 cm. long, 5 to 30 mm. broad: panicle usually strongly lobed, variable in shape, ellipsoid, cylindrical, or elongate-ovoid, apex blunt or tapering, 10.5 to 30 cm. or more long, 14 to 55 mm. in diameter; lobes variable in size and length up to 3 or 4 cm., sometimes short-pedicelled, base of panicle more or less open-interrupted: setae green, always noticeably longer than the spikelets, up to 12 mm. long: fruit yellowish to light brownish, mature usually straw, more or less rugulose, commonly obscurely so.

This form passes into subvar. *germanica* and many specimens are difficult to place.

RANGE.—Cultivated; specimens probably escapes, seen from Ontario, Canada; from Massachusetts, New York, Michigan and Texas in the United States and from Russia, British India and the Philippines.

SETARIA ITALICA (L.) Beauv. subsp. STRAMINEOFRUCTA Hubb. forma **breviseta** (Doell) comb. nov. *Panicum italicum* var. *brevisetum* Doell, Rhein. Fl. 128 (1843): Doell, Fl. Grossherz. Bad. **1**: 233 (1857) [excl. Host reference]: Körnicke, in Körn. & Wern. Handb. Getreideb. **1**: 274 (1885): Wern. in Körn. & Wern. l. c. **2**: 893 (1885). *P. italicum* var. *inerme* Doell, in Mart. Fl. Bras. 2^2: 165 (1877) cf. Körnicke, in Körn. & Wern. l. c. **1**: 274 (1885). *P. brevisetum* Doell, in Mart. l. c. 2^2: 165 (1877) in syn. sub P. italicum var. inerme. *P. italicum* var. *brevisetum* subvar. *insigne* Körnicke, in Körn. & Wern. l. c. **1**: 274 (1885): Wern. in Körn. & Wern. l. c. **2**: 894 (1885). *P. italicum* var. *brevisetum* subvar. *maximum* Körnicke, ex Wern. in Körn. & Wern. l. c. **2**: 894 (1885). *Setaria glomerato-spicata* Hort. ex Körnicke, in Körn. & Wern. l. c. **1**: 274 (1885) in syn. sub P. italicum var. brevisetum. *S. italica sibirica* Hort. ex Körnicke, in Körn. & Wern. l. c. **1**: 274 (1885) in syn. sub P. italicum var. brevisetum.

Differs from the subspecies in having the setae shorter than the spikelets or barely exceeding them.

RANGE.—Specimens seen from West Virginia and British India, also cultivated specimens from Massachusetts and Washington, District of Columbia.

SETARIA ITALICA (L.) Beauv. subsp. STRAMINEOFRUCTA Hubb. subvar. **germanica** (Mill.) comb. nov. *Panicum germanicum* Mill. Gard. Dict., ed. 8, Panicum no. 1 (1768). *Pennisetum macrochaeton* Jacq. Eclog. Gram. **3**: 36, t. 25 (1815–20). *Panicum italicum* Var. B Metzger, Eur. Cereal. 63, t. 19, f. B (1824). *Setaria macrochaeta* (Jacq.) Spreng. Syst. Veg. **1**: 305 (1825): Kunth, Enum. Pl. **1**: 152 (1833) [as to name and syn.]. *Panicum italicum* var. *praecox* Alef. Landw. Fl. 315 (1866): Körnicke, in Körn. & Wern. Handb. Getreideb. **1**: 276, t. 8, f. 41 (1885). *P. italicum* var. *macrochaetum* (Jacq.) A. Br. Ind. Sem. Hort. Berol. **1871**: 4 (1871): Doell, in Mart. Fl. Bras. 2^2: 165 (1877) [as to name, perhaps not as to plant]. *Chaetochloa italica germanica* (Mill.) Scribn. U. S. Div. Agrost. Bull. **6**: 32 (1897): Scribn. & Merr. U. S. Div. Agrost. Bull. **21**: 21 (1900) [as to name, possibly subvar. *Metzgeri* as to plant]. *Panicum italicum* stirps *germanicum* (Mill.) Dalla Torre & Sarnth. Fl. Tirol. 6^1: 158 (1906) [as to name, excl. syn. *S. germanica* Beauv.].

Differs from the subpsecies as follows,—plant usually more slender and often not so tall; leaves in general shorter and narrower; panicle variable in size, 1.8 to 13 cm. long, 8 to 15 mm. in diameter, dense or very slightly lobulate at the base, occasionally one or two small interrupted lobes, lanceolate-ellipsoid to elongate-cylindrical; setae noticeably longer than the spikelets. The extreme small forms are sometimes only 1 dm. tall and have panicles very closely resembling *S. viridis*.

RANGE.—Commonly cultivated; specimens—escapes or adventitious—seen from various parts of the United States, from Europe, Russia in Asia, British India, China and the Philippines and from Japan.

SETARIA ITALICA (L.) Beauv. subsp. STRAMINEOFRUCTA Hubb. subvar. GERMANICA (Mill.) Hubb. forma **mitis** (Alef.) comb. nov. *Panicum italicum* Var. E Metzger, Eur. Cereal. 64, t. 17, f. B (1824). *P. italicum* var. *mitis* Alef. Landw. Fl. 316 (1866): Körnicke, in Körn. & Wern. Handb. Getreideb. **1**: 277, t. 8, f. 42 (1885) [spelling emended var. *mite*]: Wern. in Körn. & Wern. l. c. **2**: 898 (1885).

Differs from the subvariety only in having the setae shorter than or barely exceeding the spikelets.

RANGE.—A single specimen from Quebec, Canada, also one from France and one from China.

A somewhat digitately forked form of subsp. *stramineofructa* is described and pictured by Rumphius, Herb. Amboin. **5**: 202, t. 75, f. 2 (1750) and is also spoken of by Körnicke, in Körn. & Wern. Handb. Getreideb. **1**: 278 (1885). This form is represented by the following specimen from Texas, *Nealley* in 1886 in U. S. Nat. Herb.

SETARIA ITALICA (L.) Beauv. subsp. STRAMINEOFRUCTA Hubb. var. **Hostii** nom. nov. *Panicum italicum* Host, Ic. Gram. Austr. **4**: 8 in part, t. 14 (1809) [the text covers much more than this form; the plate is an excellent representation of the form]. *P. germanicum* Willd. Sp. Pl. **1**: 336 (1797) fide description of specimen in Willdenow's Herbarium and fragment of the same. *Setaria italica β germanica* (Willd.) Ledeb. Fl. Ross. **4**: 471 (1853) [as to name]: Richter, Pl. Eur. **1**: 28 (1890): Beck, Fl. Nieder-Oesterr. **1**: 46 (1890) [excl. syn. var. *brevisetum* Doell]. *Panicum italicum* var. *macrochaetum* Körnicke, in Körn. & Wern. Handb. Getreideb. **1**: 273 (1885) [as to plant and descr., not as to syn.].

Differs from the subspecies principally in having purple setae:

rarely only purple-tipped. The leaves are the same as in the subspecies; the panicles are always lobulate, but sometimes more dense than in the subspecies and almost cylindrical, 7.5 to 22 cm. long, 15 to 40 mm. in diameter; the setae are noticeably longer than the spikelets, up to 18 mm. long.

This form passes into subvar. *Metzgeri* and many specimens are difficult to place.

RANGE.—Commonly cultivated. Specimens not strictly cultural seen from eastern and central United States, from Germany, Russia in Asia and from British India.

SETARIA ITALICA (L.) Beauv. subsp. STRAMINEOFRUCTA Hubb. var. HOSTII Hubb. subvar. **Metzgeri** (Körnicke) comb. nov. *Panicum germanicum* Host, Ic. Gram. Austr. **2**: 12 in part, t. 15 (1802) [the description covers more than this form, the plate is a perfect representation of the commonest strain of the subvariety]. *P. italicum* var. *Metzgeri* Körnicke, in Körn. & Wern. Handb. Getreideb. **1**: 276 (1885): Wern. in Körn. & Wern. l. c. **2**: 896 (1885). *Setaria italica* var. *Metzgeri* (Körnicke) Hack. ex Gramina Hungarica Exsiccatae **7**: no. 303[6] and in Exsiccatae List published in Magyar Bot. Lapok **10**: 462 (1911) nomen.

Differs from var. *Hostii* principally in its shorter, denser panicles [rarely slightly lobulate toward the base] which are often clavate and truncate or sometimes penicillate at the tip and frequently taper at the base owing to empty clusters of setae. Plant commonly not so tall; leaves usually shorter and narrower; panicle 1 to 13 cm. long, 7 to 17 mm. in diameter; setae always noticeably longer than the spikelets, up to 14 mm. long. Certain specimens resemble purple-setaed specimens of *S. viridis*, but are readily distinguished by the free fruit and ordinarily by the larger spikelets.

RANGE.—One of the most commonly cultivated strains and widely escaped in many parts of Canada and the United States. Specimens also seen from Italy, Hungary, Russia in Europe, India and the Philippines.

SETARIA ITALICA (L.) Beauv. subsp. STRAMINEOFRUCTA Hubb. var. HOSTII Hubb. subvar. METZGERI (Körnicke) Hubb. forma **curtiseta** forma nov.

Differt a subvarietate *Metzgeri* setis quam spiculis brevioribus vel vix superantibus.

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[6] Prof. Häckel wrote me that the combination was published on the label of this set of exsiccatae and synonymy is given on the printed label.

Differs from subvariety *Metzgeri* in having the setae shorter than barely exceeding the spikelets.

RANGE.—Only specimen see, Italy—Venetia—Patavium (Padova), Fl. Italica Exicc., ser. 2, no. 1002 [Type in Gray herb.].

SETARIA ITALICA (L.) Beauv. subsp. STRAMINEOFRUCTA Hubb. var. **brunneoseta** var. nov.

Differt imprimis a subspecie colore setarum. Setae flavidibrunneae, longitudine variabiles, ad 17 mm. longae, semper quam spiculis multo longiores. Panicula lobulata, 9–27 cm. longa, 15–30 mm. in diametro. Fructus aliquando fuscostramineus. Spiculae 2–3 mm. longae.

Differs from the subspecies chiefly in the color of the setae which are yellow brown, variable in length, but always noticeably longer than the spikelets, up to 17 mm. long. The panicle is lobulate, 9 to 27 cm. long, 15 to 30 mm. in diameter. Fruit sometimes dark straw. Spikelets 2 to 3 mm. long.

RANGE.—Philippines, Luzon, *Mangubat* in 1906, Bur. Sci. Philipp. no. 1344 [Type in Gray herb.]. I have also seen a specimen from Tennessee, *Robertson* in 1873 and several cultural specimens.

SETARIA ITALICA (L.) Beauv. subsp. STRAMINEOFRUCTA Hubb. var. BRUNNEOSETA Hubb. forma **brachychaeta** forma nov.

Varietate similis sed setae quam spiculis breviores vel eas paullo superantes. Panicula interdum pro longitudine tenuior.

Like the variety, but the setae shorter than or slightly exceeding the spikelets in length,—panicle sometimes relatively more slender.

RANGE.—India—Mont. Khasia, *Hooker & Thomson* [type in Gray herb.] and Formosa—Bankiang, *Henry*, no. 571 are the only specimens of this form I have seen.

SETARIA ITALICA (L.) Beauv. subsp. STRAMINEOFRUCTA Hubb. var. BRUNNEOSETA Hubb. subvar. **densior** subvar. nov.

Differt imprimis a varietate panicula densiore compactiore elobata vel paululim ad basin lobata, 4–15 cm. longa, 8–23 mm. in diametro. Folia saepe grandia usque 40 cm. longa, 37 mm. lata. Fructus interdum fusco-stramineus.

Differs from the variety chiefly in the denser more compact panicle which is not lobed or very slightly so at the base, 4 to 15 cm. long, 8 to 23 mm. in diameter. The leaves frequently large up to 40 cm. long, 37 mm. broad. The fruit occasionally dark straw.

RANGE.—Massachusetts—Weston, *Williams* in 1895 [Type in

Gray herb.]. I have also seen specimens from Maine, Louisiana, Russia in Asia and China, besides several cultural specimens.

SETARIA ITALICA (L.) Beauv. subsp. **rubrofructa** nom. nov. *Panicum, italicum* var. *erythrospermum* Körnicke, Syst. Übers. Cereal. 19 (1873) [not *P. erythrospermum* Vahl, ex Hornem.]: Körnicke, in Körn. & Wern. Handb. Getreideb. **1**: 274 (1885): Wern. in Körn. & Wern. l. c. **2**: 892 (1885).

This form according to Körnicke, in Körn. & Wern. l. c. **1**: 274 (1885) has a long slender, rather open, lobulate panicle up to 21 cm. long and 16 mm. in diameter; the setae are green and very long; the fruit intensive light red, shiny and is said to be small; the last statement according to Werner, in Körn. & Wern. l. c. **2**: 892 (1885).

NOTA.—Körnicke names this form *Panicum italicum* var. *erythrospermum*, but it is inadvisable to take up this name as it is apt to lead to confusion owing to *P. erythrospermum* Vahl, ex Hornem. Hort. Hafn. **1**: 82 (1813) which has purple setae and is referable to subvar. *violacea*.

RANGE.—I have seen no specimens; Körnicke says from Botanic Gardens.

SETARIA ITALICA (L.) Beauv. subsp. RUBROFRUCTA Hubb. forma **gigas** (Körnicke) comb. nov. *Panicum italicum* var. *gigas* Körnicke, in Körn. & Wern. Handb. Getreideb. **1**: 275 (1885): Wern. in Körn. & Wern. l. c. **2**: 896 (1885). *P. frumentaceum* Hort. ex Körnicke, in Körn. & Wern. l. c. **1**: 275 (1885) in syn. sub P. italicum var. gigas.

Körnicke, in Körn. & Wern. l. c. **1**: 275 (1885) describes this form as having a very large and thick panicle up to 23 cm. long and 30 mm. in diameter. Setae less numerous and scarcely exserted: green [cf. Key on page 272]. Fruit dark red.

RANGE.—I have seen no specimens; Körnicke says he received it from Hungary as *Panicum frumentaceum* and that this strain was derived from the Austrian East-Indian expedition.

SETARIA ITALICA (L.) Beauv. subsp. RUBROFRUCTA Hubb. subvar. **pabularis** (Alef.) comb. nov. *Panicum italicum* Var. C Metzger, Eur. Cereal. 64 (1824). *P. italicum* var. *pabularis* Alef. Landw. Fl. 315 (1866): Körnicke, in Körn. & Wern. Handb. Getreideb. **1**: 276 (1885) [emended spelling var. *pabulare*].

This form is described by Körnicke, in Körn. & Wern. l. c. **1**: 276 (1885) as belonging to the *Moharium* group,—that is small, dense-panicled,—having green setae and orange-yellow fruit.

RANGE.—I have seen one cultivated specimen from Europe which

I have tentatively referred to this form; Körnicke says that this form is unknown to him, but that it has been experimentally cultivated in Germany and France.

SETARIA ITALICA (L.) Beauv. subsp. RUBROFRUCTA Hubb. var. **purpureoseta** var. nov.

Differt a subspecie setis fusco-purpureis. Folia 30 cm. longa, 20 mm. lata. Panicula crebre lobulata ad basin interrupta, 23 cm. longa, 20 mm. in diametro; setae longae, purpureae. Fructus rubro-aurantiacus.

Differs from the subspecies principally in having purple setae. Leaf 30 cm. long, 20 mm. broad. Panicle close-lobulate, base interrupted, 23 cm. long, 20 mm. in diameter. Setae longish, purplish. Fruit red-orange.

RANGE.—India—Himalayan Herbarium *Strachey and Winterbottom*, no. 3 [Type in Gray herb.]. I have also seen cultural specimens in the Economic Collection of the U. S. Bur. Pl. Ind.

SETARIA ITALICA (L.) Beauv. subsp. RUBROFRUCTA Hubb. var. PURPUREOSETA Hubb. subvar. **violacea** (Alef.) comb. nov. *Panicum erythrospermum* Vahl, ex Hornem. Hort. Hafn. **1**: 82 (1813): Jacq. Eclog. Gram. **3**: t. 24 (1815–20) [setae represented more pink than usual]. *Echinochloa erythrosperma* (Vahl) R. & S. Syst. Veg. **2**: 477 (1817). *Pennisetum erythrospermum* (Vahl) Jacq. Eclog. Gram. **3**: 34 (1815–20). *Panicum italicum* Var. D Metzger, Eur. Cereal. 64 (1824). *Setaria erythrosperma* (Vahl) Spreng. Syst. Veg. **1**: 304 (1825) [excl. syn. *laevigatum*]. *Panicum italicum* var. *violaceum* Alef. Landw. Fl. 316 (1866): Körnicke, in Körn. & Wern. Handb. Getreideb. **1**: 276 (1885). *P. italicum* var. *erythrospermum* (Horn.) A. Br. Ind. Sem. Hort. Berol. **1871**: 4 (1871).

Panicle dense, often somewhat lobulate and interrupted at the base, but empty; 8 to 13 cm. long, 10 to 15 mm. in diameter. Leaves usually narrower and plant probably more slender and not as tall [specimens examined run from 5 to 8.5 dm. tall]. Setae purple or sometimes only purple-tipped, noticeably longer than the spikelets, but rather short, up to about 7 mm.

RANGE.—The only specimens of this form which I have seen are cultural.

SETARIA ITALICA (L.) Beauv. subsp. RUBROFRUCTA Hubb. var. **rubra** (Körnicke) comb. nov. *Panicum italicum* var. *rubrum* Körnicke, in Körn. & Wern. Handb. Getreideb. **1**: 274 (1885): Wern. in Körn. & Wern. l. c. **2**: 892 (1885).

Varies from the subspecies mainly in having brown setae which are short, but at least twice the length of the spikelets about 6 mm. long. Panicle rather slender, lobulate, somewhat loosely so and interrupted at the base, 13 to 17 cm. long, 13 to 15 mm. in diameter. Spikelets small. Fruit orange-brown.

This form undoubtedly passes into subvar. *condensa*.

RANGE.—I have seen specimens from Assam and the Philippines.

SETARIA ITALICA (L.) Beauv. subsp. RUBROFRUCTA Hubb. var. RUBRA (Körnicke) Hubb. forma **aurantiaca** (Körnicke) comb. nov. *Panicum italicum* var. *aurantiacum* Körnicke, in Körn. & Wern. Handb. Getreideb. **1**: 275 (1885): Wern. in Körn. & Wern. l. c. **2**: 895 (1885). *Setaria persica* Hort., *S. brevifolia* Hort., *Panicum macrurum* Hort., and *P. eriogonum* Hort. all ex Körnicke in Körn. & Wern. l. c. **1**: 275 (1885) sub P. italicum var. aurantiacum.

Setae shorter than or barely exceeding the spikelets; panicles 9 to 10 cm. long, 8 to 10 mm. in diameter, otherwise like the variety.[7]

RANGE.—Only specimen examined, Philippines—Luzon, *Ramos* in 1909, Bur. Sci. Philipp. no. 7842. Körnicke mentions the form from Algeria.

SETARIA ITALIACA (L.) Beauv. subsp. RUBROFRUCTA Hubb. var. RUBRA (Körnicke) Hubb. subvar. **condensa** subvar. nov.

Differt a varietate panicula breviore non lobata.

This form differs from the variety in having shorter, dense, not-lobed panicles, with some scattered empty tufts of setae below the dense portion: measurements without these tufts 4.8 to 6.5 cm. long, about 10 mm. in diameter. Plant about 5 dm. tall and rather slender: leaves smaller and narrower. Fruit rather more orange-red than the variety.

RANGE.—Only specimen seen, Assam, *Chatterjee* in 1902[8] [type in Gray herb.].

SETARIA ITALICA (L.) Beauv. subsp. **nigrofructa** nom. nov. *Panicum italicum* var. *nigrum* Körnicke, in Körn. & Wern. Handb. Getreideb. **1**: 274 (1885): Wern. in Körn. & Wern. l. c. **2**: 893 (1885).

According to Körnicke, in Körn. & Wern. l. c. **1**: 274 (1885) this form has thick panicles somewhat shorter and less lobulate than *Panicum italicum* var. *erythrospermum* Körnicke—which is a synonym of subsp. *rubrofructa*—; has green setae of medium length; the fruit mottled and violet-black.

---

[7] Körnicke, in Körn. & Wern. l. c. **1**: 275 (1885) gives somewhat larger measurements—panicle up to 17 cm. long, and up to 20 mm. in diameter.

[8] Material of this collection in the U. S. Nat. Herb. is var. *rubra*.

RANGE.—I have seen no specimens; Körnicke reports it from Hungary.

SETARIA ITALICA (L.) Beauv. subsp. NIGROFRUCTA Hubb. var. **atra** (Körnicke) comb. nov. *Panicum italicum* var. *atrum* Körnicke, Syst. Übers. Cereal. 19 (1873): Körnicke, in Körn. & Wern. Handb. Getreideb. **1**: 277 (1885): Wern. in Körn. & Wern. l. c. **2**: 899 (1885).

Setae brownish-purple, noticeably longer than the spikelets, up to 7 to 8 mm. long. Panicles dense, occasionally somewhat lobulate and interrupted at the base, 1.5 to 10 cm. long, 7 to15 mm. in diameter, frequently with clusters of empty setae below the dense portion of the panicle. Fruit brown-black to black when mature and well developed. [There are always present in larger or smaller proportion less well developed pale-straw colored fruits.]

RANGE.—All the specimens which I have seen are cultural.

The cultural names applied to the various forms of *S. italica*—collectively known as Foxtail Millets—often cover more than one form of the variants. Sometimes no distinction is made in setae color sometimes the density of the panicle is not a factor. Certain strains in cultivation have been given names and frequently these names seem to have been used for more than one strain. For the application of the following cultural names I am indebted to Mr. H. N. Vinall and Mr. M. A. Carleton of the U. S. Dept. of Agriculture.

GERMAN MILLET.—A large lobed form with long setae, green or purple in color, no distinction being made. Applies indiscriminately to subsp. *stramineofructa* and var. *Hostii*. Cf. Carleton, in Bail. Cycl. Am. Agric. **2**: 469, f. 695 (1907).

GOLDEN WONDER MILLET.—A large lobed form with setae shorter than or barely exceeding the spikelets. Applies to forma *breviseta*.

COMMON MILLET.—A compact form with dense spike-like panicles of the *Moharium* group. The setae are long, but no distinction of color is made. Applies indiscriminately to subvar. *germanica* and subvar. *Metzgeri;* probably also to stout-panicled, large-fruited specimens of subvar. *densior*. Cf. Carleton, in Bail. l. c. **2**: 469, f. 694 & 470, f. 697 (1907).

AINO MILLET.—Sometimes termed Japanese Millet, a misleading name, as it covers various strains. Commonly a slender lobulate form with brown setae and small fruit, about 2 mm. long, but also used for slender, small-fruited, compact-panicled specimens. Cultivated by the Ainos of Japan and also occurring in the Philippines. Applies to the slender-panicled, small-fruited specimens of var. *brunneoseta* and to similar specimens of subvar. *densior*. Cf. Carleton, in Bail. l. c. **2**: 469, f. 696 (1907).

TURKESTAN MILLET.—A robust growing form with lobulate panicles, orange or reddish fruit and purple setae. Similar to Kursk Millet, but much coarser and ripening later. Applies to var. *purpureoseta*.

KURSK MILLET.—Sometimes called Russian Millet, but this term seems to have been used also for the Siberian Millet. A form, belonging to the *Moharium* group, with dense or at base slightly lobed panicles, red or orange fruit and long, purplish setae. Said to be the result of breeding selection from Siberian Millet. Applies to subvar. *violacea*.

SIBERIAN MILLET.—A form with large, more or less open lobulate panicles, orange or red fruit and long, brown setae. Applies to var. *rubra*, but judging by the figure under the name Red Siberian Millet it has been also used for subvar. *violacea*, cf. Carleton, in Bail. l. c. **2**: 469, f. 693 (1907).

HUNGARIAN MILLET or MOHAR.—Also called Hungarian Grass. A form, belonging to the *Moharium* group, with dense, spike-like panicles, fruit blackish or brownish-black with pale yellowish-straw fruits intermingled (these sometimes predominating) and long purplish-brown setae. The panicles average smaller than those of the other forms commonly cultivated [a small strain of subvar. *Metzgeri* is apt to be as small]. Applies correctly to var. *atra*, but seems to have been used also for subvar. *Metzgeri*, cf. Carleton, in Bail. **2**: 470, f. 698 (1907).

The following combinations have commonly been refered as synonyms to *Setaria italica*, but I believe that they are better referable to other species.

Panicum indicum Mill. Gard. Dict., ed. 8, Panicum no. 3 (1768) [cited by Hook. f. Fl. Brit. Ind. **7**: 78 (1896) as a synonym of *S. italica*. The Miller description points to *Pennisetum americanum* (L.) Leeke and the Bauhin reference given by Miller is certainly *P. americanum*. Mr. A. B. Rendle writes me that there is no specimen of Panicum indicum in the Miller herbarium].

P. laevigatum Muhl. Cat. 9 (1813) nomen: Muhl. in Ell. Sk. **1**: 112

(1816): Muhl. Descr. Gram. 100 (1817) [I believe this is undoubtedly a form of *S. imberbis*, cf. Scribn. & Merr. U. S. Div. Agrost. Bull. **27**: 2 (1900)].

Setaria rubicunda Dum. Obs. Gram. Belg. 139 (1823) [this is referred to *S. italica*, but seems better referable to *S. glauca*, cf. Hook. f. Fl. Brit. Ind. **7**: 79 (1896)].

S. multiseta Dum. l. c. 138 (1823) [referred in synonymy to *S. italica* by Richter, Pl. Eur. **1**: 28 (1890), but I believe it is more probably *S. glauca* though "setae . . . deorsum scabrae" does not fit *S. glauca* and does apply to *S. verticillata*].

Panicum viride var. *rubicundum* (Dum.) Lej. Rev. Fl. Spa 217 (1824): Lej. & Court. Comp. Fl. Belg. **1**: 51 (1828) [cf. *Setaria rubicunda* Dum. on which it is based].

Setaria viridis var. *rubicunda* (Dum.) Th. Dur. in Wildem. & Dur. Prodr. Fl. Belg. **3**: 80 (1900) [Durand did not publish the variety in Cat. Fl. Liége as he states, in Prodr. Fl. Belg., that he did: cf. *S. rubicunda* Dum.].

S. viridis var. *multiseta* (Dum.) Th. Dur. in Wildem. & Dur. Prodr. Fl. Belg. **3**: 80 (1900) [Durand did not publish the variety in Cat. Fl. Liége as he states, in Prodr. Fl. Belg., that he did: cf. *S. multiseta* Dum. on which it is based].

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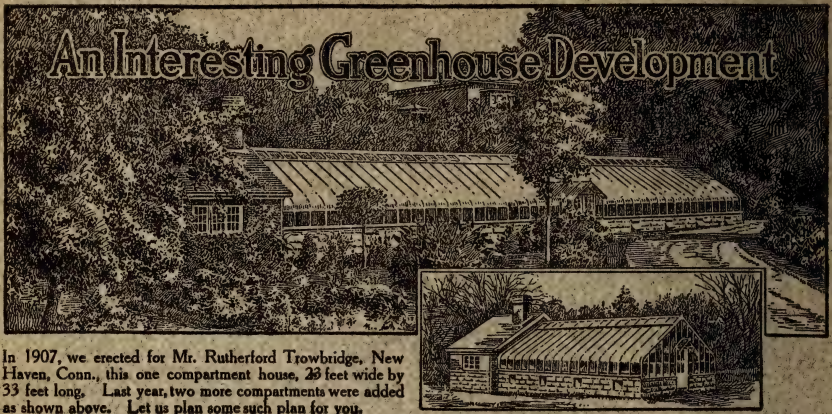

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THE MORPHOLOGY AND SYSTEMATIC POSITION OF PODOMITRIUM

Douglas Houghton Campbell

The present classification of the thallose Jungermanniales ("Anacrogynae" of Leitgeb) is very far from satisfactory, as our knowledge of the morphology of several genera is still very fragmentary.

The genus Podomitrium, with which the present paper is concerned, comprises two known species, *P. Phyllanthus* of the Australasian region, and *P. Malaccense*, which Stephani[1] states has been reported hitherto only from Singapore and New Caledonia. Stephani unites Podomitrium with Umbraculum to form the genus Hymenophytum, but there seems to be ample reason for separating the two genera.

In the spring of 1913 the writer collected at several places in Sarawak, in Western Borneo, abundant material of *P. Malaccense*, which is a common liverwort of this region, and it was also found at a single station in Luzon, Philippine Islands. It is highly probable that the plant will be found in the Southern Philippines also, but, as the sterile plants are indistinguishable from a Blyttia, it cannot be certainly recognized except when fertile.

Podomitrium Malaccense grows upon the ground, usually upon clay soil, and, when it was first collected by the writer, it was supposed to be a species of Blyttia, which it exactly resembles except for the special fertile branches developed from the ventral side of the thallus. In the species under consideration the thallus is usually very unsymmetrical, one wing being almost completely suppressed (figs. 1, *B*; 3, *A*). The conspicuous midrib bears upon its lower surface numerous short rhizoids which are generally in bunches separated by considerable areas nearly or quite bare of rhizoids (fig. 1, *A*).

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[1] Stephani, F. Hymenophytum Malaccense St. Species Hepaticarum—Mém. Herb. Boiss. **11**, 1900.

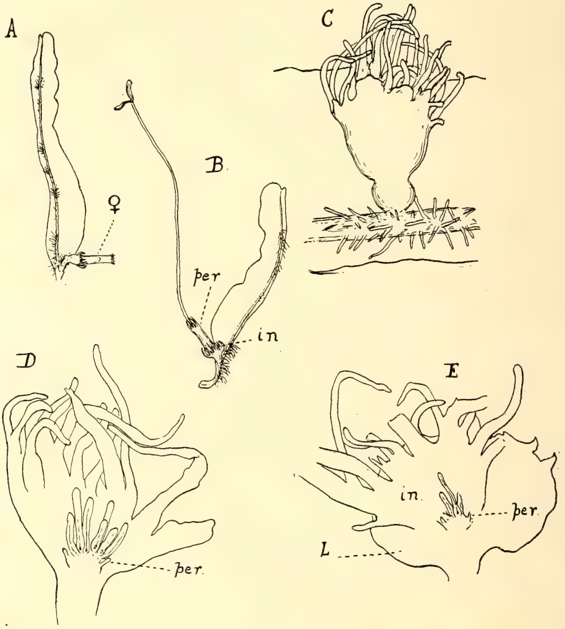

FIG. 1. *A*. Female plant of *Podomitrium Malaccense* seen from below, showing the branch ♀ bearing a young sporophyte, × 2. *B*. A plant bearing a ripe sporophyte which has opened by two free valves; the base of the seta is enclosed in the perianth, *per*, and the involucre, *in*. *C*. A young archegonial branch seen from below, × 16. *D*, *E*. A somewhat older branch split through the middle of the archegonial group—*L*, lamina of the shoot; *in*, the involucre; *per*, the undeveloped perianth.

The anatomy of the thallus is almost identical with that of Blyttia. The clearly defined midrib is composed of several thicknesses of small cells while the lamina has but a single layer of larger cells. Traversing the midrib is a strand of narrow, thick-walled, pitted cells, closely resembling the corresponding tissue in Blyttia (fig. 2, *C*, *D*).

As in other Jungermanniales the growth of the shoot is from a single apical cell which closely resembles that found in most species of Blyttia.[2] It is the common "two-sided" apical cell, with segments cut off successively right and left. In horizonatal section it has the form of a rather narrow isosceles triangle, while in vertical longitudinal section it appears as a nearly equilateral triangle (fig. 2, *A*, *B*).

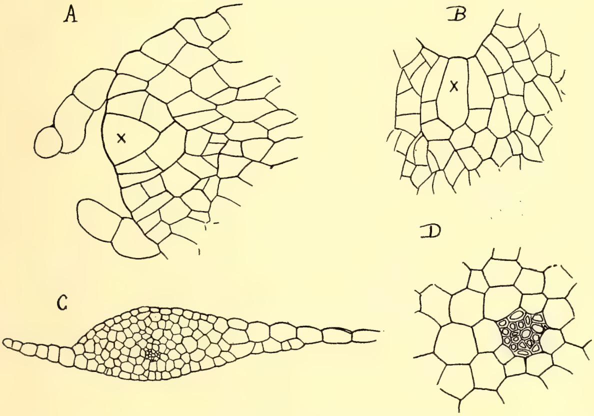

FIG. 2. *A*. Vertical longitudinal section through the apex of the thallus, showing the single apical cell, *x*; × about 300. *B*. Horizontal section of the thallus apex. *C*. Cross-section of the thallus, showing the very unequal wings, and the massive midrib with its strand of conducting tissue; × about 35. *D*. The central region of the midrib; × about 200.

In the position of the sexual organs Podomitrium most nearly resembles Metzgeria in having them borne upon special short branches which arise from the flanks of the midrib upon the ventral side of the thallus. These special fertile branches at once distinguish Podomitrium from Blyttia where both archegonia and antheridia are borne upon the unmodified thallus. The antheridia are borne upon short flattened branches which may be produced on both sides of the midrib of a main shoot, or, in case the lamina of the latter is suppressed, on one

[2] Campbell, D. H. and Williams, F. A Morphological Study of Some Member of the Genus Pallavicinia. Stanford University, 1914.

side; the antheridial branches are developed from the other side only of the main shoot (fig. 3, *A*).

While the antheridia are produced for the most part in two rows corresponding to the two sets of segments cut off from the apical cell some of them may be formed almost in the median line of the shoot, so that an imperfect third row may be present. Each antheridium is covered by a scale with irregularly laciniate margin, and the lamina of the antheridial shoot also has its margins irregularly toothed and lobed (fig. 3, *C*). In the arrangement of the antheridia, *Podomitrium Malaccense* recalls very strongly *Blyttia* (*Mittenia*) *Zollingeri*,[3] and perhaps even more *Calycularia radiculosa* (Sande Lac) Steph.[4]

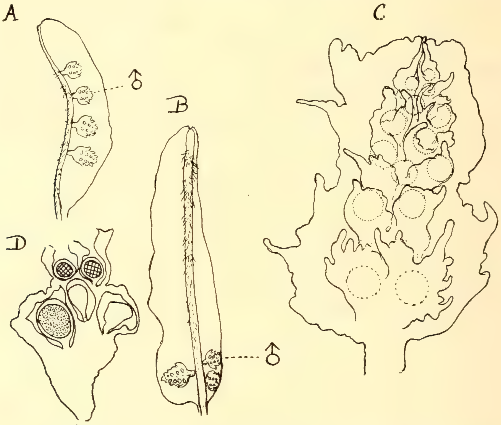

FIG. 3. *A*. Male plant, with the lamina on one side entirely suppressed; ♂, the antheridial branches; × 2. *B*. Male plant with antheridial branches developed on both sides of the midrib. *C*. An antheridial branch, showing the antheridia covered by the subtending scales; × 25. *D*. Horizontal section of a very young antheridial branch, × about 35.

The youngest antheridia occur close to the growing point of the shoot. The beginning of the subtending scale is evident as soon as the antheridium can be recognized (fig. 4, *A*). The development of the antheridium is almost exactly like that of Blyttia, and indeed does not show any marked departure from the type characteristic of the Jungermanniales. The mother-cell of the antheridium divides first

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[3] Campbell and Williams, loc. cit.

[4] Campbell, D. H. The Morphology and Systematic Position of Calycularia radiculosa (Steph.). Reprint from Dudley Memorial Volume, Stanford University, 1913.

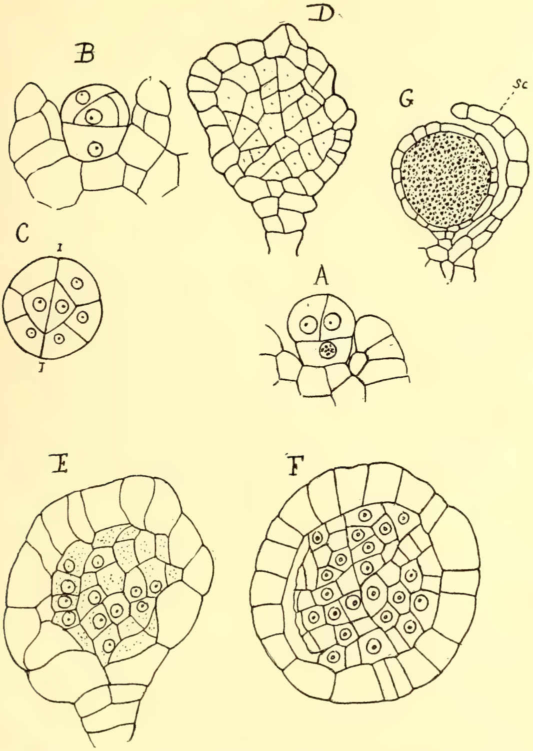

FIG. 4.—*A*, *B*. Very young antheridia, seen in longitudinal section. *B* is cut in a plane at right angles to that of *A*; × 335. *C*. Cross-section of a somewhat older stage, × 335; *I–I* the primary median wall. *D*. Nearly median section of an older antheridium. *E*, *F*. Two sections, slightly oblique of an older stage. *G*. Median section of a ripe antheridium, × 75. *sc*, the subtending scale.

into a short basal cell which subsequently divides further to form the short stalk of the older antheridium, and a terminal cell which is nearly hemispherical and develops into the body of the antheridium. This hemispherical cell, as usual in the Jungermanniales, first divides by a vertical median wall, and in each of the two cells thus formed two intersecting walls next arise which separate an inner cell in contact with the original median wall, and two peripheral cells (fig. 4, *B*). In cross section, the pair of central cells, from which subsequently the sperm-cells arise, appear triangular in outline (fig. 4, *C*). As in most other Jungermanniales, the peripheral cells divide by radial walls only, so that the wall of the antheridium remains permanently but a single cell in thickness. The further divisions in the central cells were not followed in detail but the final divisions result in the spermatocytes which are in pairs as has been described in various other Hepaticae. The details of spermatogenesis were not critically studied, as the material did not seem to be especially favorable for this purpose, and there was

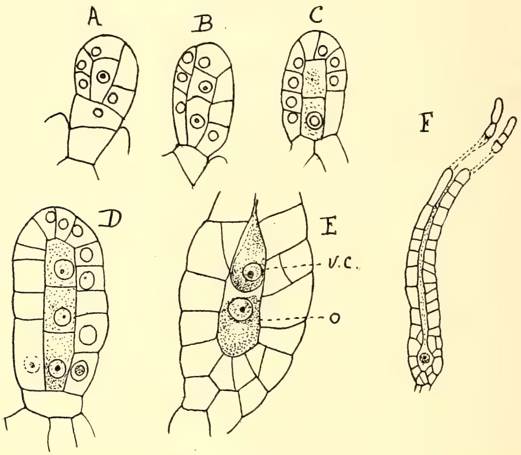

FIG. 5. *A–E.* Development of the archegonium, seen in longitudinal section; × 335. *F.* An old archegonium, which has not been fertilized; × 75. *v. c.*, ventral canal cell; *o*, egg cell.

nothing to indicate any deviation from what has been described for other related forms. The number of chromosomes in the sperm nucleus is eight, the same as has been found in Blyttia.

The archegonia are in a group upon the dorsal surface of short lateral shoots (fig. 1, *C–E*). These archegonial branches are so short that they are almost hidden by the large involucre which surrounds the archegonial group. No midrib is developed in the archegonial shoot which has the form of a thin nearly orbicular lamina with a more or less conspicuously toothed margin. The involucre has its margin composed of numerous slender appendages forming a dense fringe. The archegonia may number a dozen or more in a receptacle, and show different ages in the same group. They are borne upon a more or less evident elevation or placenta. Surrounding each group of archegonia is an inconspicuous elevated ridge or ring—the perianth. Should none of the archegonia be fertilized the perianth remains rudimentary; but, where an embryo is developed, the perianth grows very rapidly and forms a tubular sheath which encloses the growing sporophyte and projects far beyond the involucre (fig. 1, *A*, *B*). The margin of the perianth, like that of the involucre, is conspicuously fringed.

Both involucre and perianth in Podomitrium are very much like those of Blyttia, and, except that the archegonia are produced on special branches, the two genera are scarcely distinguishable. The archegonia themselves (fig. 5) show no marked peculiarities, and are also practically identical in structure with those of Blyttia. There is usually a more or less evident stalk developed, and in the terminal cell of the young archegonium there are formed the usual three intersecting walls cutting out an axial cell from three peripheral cells. From the former the cap cell is now cut off, and this sooner or later divides by quadrant walls into the usual four terminal cells of the archegonium. This division generally takes place somewhat later in Podomitrium than is usual in the Liverworts, and sometimes it looks as if there was a limited apical growth of the young archegonium due to lateral segments cut off from the primary cap cell before its final division by quadrant walls (fig. 5, *D*). In this respect *Podomitrium Malaccense* may be compared with *Blyttia Levieri*[5], where a similar condition has been noted. It also recalls the apical growth in the moss archegonium, although, in the latter, canal cells are also cut off from the basal segments of the cap cell, while such limited apical growth as may occur in Podomitrium and some other Liverworts is confined to the peripheral neck cells.[6]

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[5] Campbell and Williams, loc. cit.

The transverse walls which separate the ventral and neck regions of the young archegonium are formed before any division takes place in the cap cell. The neck may become much elongated, especially when fertilization fails to occur (fig. 5, *F*).

As usual the lower of the primary axial cells divides the egg and ventral canal-cell (fig. 5, *E*), and from the other, by a succession of divisions, the row of neck canal cells is formed. The exact number of the latter could not be made out with certainty, but is probably eight.

In the ventral region, occasional periclinals may form in the peripheral cells, so that at the time of fertilization the wall of the venter is incompletely two-layered (fig. 5, *E*).

The Embryo

Most of the archegonia fail to be fertilized and only a small number of young embryos could be found in the material collected. To judge from the few that were secured, the early divisions seem to resemble very much those in the embryo of Blyttia. Figure 6, *A*, shows the youngest embryo that was found. This was somewhat shrunken but showed an apparent division into two cells by means of an obliquely transverse wall. This first wall is probably followed by one or two more transverse walls before any vertical divisions are formed, but embryos showing these stages were not seen. The basal cell of the young embryo, and probably also the cell above it, give rise to a conspicuous appendage or haustorium, which, however, is not so large as in Blyttia.

Fig. 6, *B*, shows two nearly median longitudinal sections of an older embryo in which all but the lowest of the primary cells had divided by median vertical walls. In the upper regions there were also secondary transverse divisions, but as yet no periclinal walls.

No stages were encountered between the one just described and very much older ones in which the sporogenous tissue was already evident, and it is impossible to say just what is the relation of the different parts of the young sporophyte to the primary divisions of the embryo. It is certain, however, that the basal cell, and probably

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[6] Gayet, L. A. Recherches sur le devéloppement de l'archégone chez les muscinées. Ann. Sci. Nat. Bot. VIII. 3. 1897.

the one above it, contribute only to the haustorium, while all of the sporophyte itself—foot, seta and capsule—arise from the terminal segment, or segments.

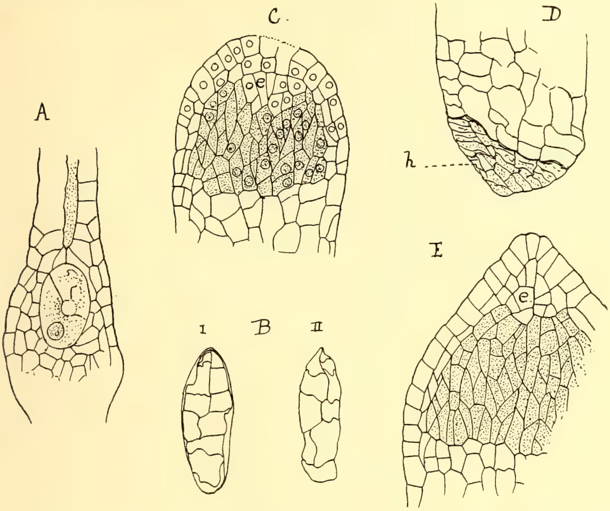

FIG. 6. *A*. Venter of archegonium containing a two-celled embryo; × about 175. *B*. Two sections of an older embryo; × 175. *C*. Upper part of older embryo showing the sporogenous tissue and the elaterophore, *e*. *D*. Base of young sporophyte, showing the remains of the haustorial tissue, *h*. *E*. Upper part of young sporophyte, more advanced than that shown in *C*.

The young sporophyte is almost cylindrical in form, and in the upper part, which is only slightly enlarged, the sporogenous tissue can be readily distinguished (fig. 6, *C*). The capsule wall at this stage is composed for the most part of two layers of cells which are decidedly larger in the apical region. Below the apex is a group of cells belonging to the sporogenous region which differ from the true sporogenous tissue in having less dense contents. This sterile mass of potential sporogenous tissue is evidently the homologue of the elaterophore found in Aneura and Metzgeria, and in this respect Podomitrium differs from Blyttia which it otherwise closely resembles. At this stage a definite foot cannot be distinguished, but the lower part of the stalk is occupied by a comparatively large mass of cells (fig. 6, *D*, *h*) which are partially disorganized, and probably represent the remains of the haustorium; but whether all of these cells are derived from the division of the one or two primary haustorial cells, could not be determined.

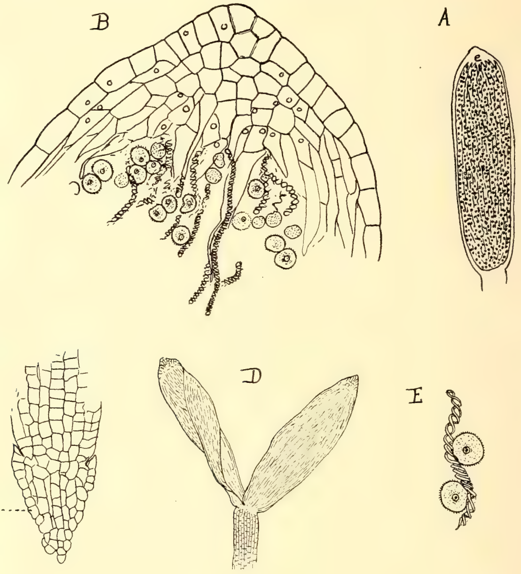

FIG. 7. *A*. Median section of a ripe capsule; *e*, elaterophore; × about 16. *B*. Upper part of the same more highly magnified. The lower cells of the elaterophore are much elongated. *C*. Foot of a nearly ripe sporophyte; × 60. *D*. Open capsule, showing the two free valves; × about 16. *E*. Sections of two ripe spores and an elater; × about 300.

Fig. 6, *E*, shows the capsule of an older sporophyte. The apex forms a decided beak as it does in Blyttia. The sporogenous tissue does not yet show an evident difference between the future spore mother cells and the young elaters.

Somewhat later, some of the cells of the sporogenous tissue divide transversely into short rows of iso-diametric cells, the young spore mother-cells, while the others remain undivided and lengthening still more finally develop into the elaters. No spore mother cells were found in division, so that the details of spore division could not be studied.

The sporophyte, as it grows older, has the foot distinctly separated from the seta by an evident constriction (fig. 7, *C*). In this respect Podomitrium more nearly resembles Mörkia or Calycularia than it does a typical Blyttia, where a distinct foot is absent. In *Blyttia* (*Mittenia*) *Zollingeri*, however, a foot like that of Podomitrium occurs.

The capsule of the ripe sporophyte closely resembles that of Blyttia from which it differs mainly in the presence of an elaterophore. The latter structure is apparently better developed in *Podomitrium Malaccense* than it is in *P. Phyllanthus* to judge from the figures of Andreas.[7] There is no doubt that in the former it originates from the sporogenous area much as it does in Aneura. The inner cells of the elaterophore are prolonged into slender processes, and these elongated cells resembled in form the elaters, but none of those that were examined showed the spiral thickenings characteristic of the true elaters.

The outer layer of cells, forming the wall of the capsule, have their cell-walls uniformly thickened as in Blyttia.

The ripe spores are about 15 μ to 17.5 μ in diameter. The outer membrane is marked by fine reticulations, thus very much resembling the spores of *Blyttia radiculosa*, whose spores are almost identical in appearance with those of *Podomitrium Malaccense* except that they are slightly smaller. According to both Andreas[8] and Cavers[9] the sporogonium of *P. Phyllanthus* opens by four longitudinal slits as it does in Blyttia; but all of the open sporogonia of *P. Malaccense* seen by the writer showed two broad valves completely separated at the top (fig. 7, *D*), resembling in this respect *Calycularia radiculosa* or Mörkia. It may be that an examination of a larger number of specimens might show some variation in the mode of dehiscence.

Comparing *Podomitrium Malaccense* with other Anacrogynae, it is evident that it comes nearest to Blyttia, from which it differs, however, in the position of the reproductive organs, the presence of a definite foot in the sporophyte, and in the presence of a distinct elaterophore, as well as a different method of dehiscence.

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[7] Andreas, J. Ueber den Bau der Wand und die Oeffnungsweise des Lebermoossporogons. Flora **86**: 1899.

[8] Andreas, loc. cit.

[9] Cavers, The Inter-relationships of the Bryophyta—reprint from New Phytologist. 1911.

A comparison of the genera included in the families Aneuraceae and Blyttiaceae, viz., Metzgeria, Aneura, Umbraculum, Podomitrium, Calycularia, Mörkia, Makinoa, Symphyogyna and Blyttia, shows that there are no constant features which can be used certainly to distinguish the two families from each other; it will probably be better, as Cavers has suggested, to combine the two families into a single one.

ON THE RELATION OF ROOT GROWTH AND DEVELOPMENT TO THE TEMPERATURE AND AERATION OF THE SOIL

W. A. Cannon

There is great diversity in root development in desert plants. This is to be seen even in a single habitat where all of the species are apparently subject to similar environmental conditions. At the same time the same species, when growing in another habitat which differs as much as may be from the first one, may, however, still retain each its characteristic type of root-system. From these general facts it appears probable that there are environmental conditions, to a degree apart from the soil *per se*, which are held in common by otherwise unlike habitats, to which the developing roots react in characteristic manner and which thus may be of determinate importance in shaping the direction of root development. Among the most striking of such common factors, which fortunately lend themselves conveniently to experimentation, are soil aeration and soil temperature. Accordingly, in the preliminary experiments looking to a solution of the general problem relating to differences in root development, the reaction of roots to aeration and to temperature were taken up.

The reaction of roots to soil aeration was studied as an introduction to this work. It is apparent that such root-systems as lie close to the surface of the soil, as, for example, that of *Fouquieria splendens*, and especially *Opuntia versicolor* and other cacti, must hold a very different relation to the atmospheric air than such root-systems as are deeply placed. And, in fact, the cultures showed that the roots of *Opuntia* had a very definite reaction to an abundant supply of air. The possible bearing of these results on the placing of the roots in the soil, or on their typical development, will be treated in the concluding section of this paper. In the meantime, it will be sufficient to state that the results of the direct aeration experiments were not entirely consistent, for whatever reason, and they will be repeated at a future time. The attention, therefore, was turned, for the time, to a study of the reaction of roots to various soil temperatures and to a consideration of the results as factors in the development of root types and in the distribution of the species.

Temperature of the Soil

The soil acts as a reservoir of heat due to the circumstance that there is usually less loss by radiation during the night than accumulates in the soil by day. It is, thus, a great temperature stabilizer. It is owing to these two facts that, to a large degree, soil temperatures are so important to the growth of the roots of plants, and, also, to plant growth in general.

As is well known the temperature of the soil varies considerably, especially with the depth beneath the surface. In the colder seasons, however, the superficial soil may register a lower temperature than the deeper soil. The amplitude of the daily and the seasonal variations in temperature, also, varies inversely as the depth. From the last condition it happens therefore that such root-systems as extend close to the surface are subject to maximum temperature changes, and, in this particular, their temperature relations form a marked contrast to the relations of the roots which lie deeply.

Among the factors which directly affect the soil temperature are its color, moisture content, and slant of the surface with respect to the position of the sun. Of these, the most important is the moisture content, for the reason that water has a specific heat about five times greater than the specific heat of the solid constituents of the soil.[1] In a later paragraph will be given a striking example of the lowering of soil temperature as a result of moistening by the summer rains. (See also, fig. 2.)

Although no extended studies have been made in the vicinity of the Desert Laboratory on exposure as a direct cause of temperature variation in soils it is clear that this factor is an important one and its importance is emphasized by the fact that the soil of the desert is poorly protected by vegetation. That the principle is of much significance can be easily shown. For example, having given a shaft of heat rays of a certain total energy E, and of a certain area A, the amount of energy impinging upon the surface of the soil will amount to E if the surface is of A extent, and this occurs when the surface lies at right angles to the incident rays. If the surface is at another angle than 90° the area is greater than A, and, for that reason, the amount of heat falling on a unit area would be less than *E*. Should, for instance, the surface lie at an angle of 45° to the incident rays, the area would be approximately 1.4 *A*, although there would be no increase in *E*, so that the amount of heat falling upon a unit area, in this instance, would be only about 70.7 per cent. of the maximum, that is, of the amount which would impinge on the surface were it at right angles to the source of heat. In short, it can be advanced that

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[1] Free, E. E., Studies in soil physics. Plant World 14: 188. 1911.

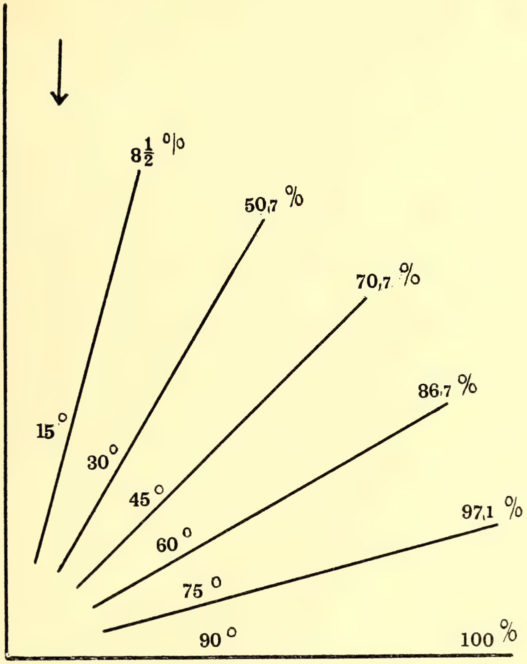

FIG. 1. Relative amount of heat per unit area falling upon the soil whose surface lies at the various angles given to the incident rays. The maximum amount received is that of a surface which is at right angles to the rays, as is indicated in the figure. The direction of the incident rays is that shown by the arrow.

the amount of heat falling upon the surface of the soil decreases with the decrease of its angle to the incident rays. The theoretical efficiency of different exposures, for several angles, is given in figure 1. It should be noted, however, as before suggested, that the amount of heat actually affecting the soil may hold different relations from that shown in the figure, for various reasons, some of which have been presented. In addition to these, it can be said, that the angle with the vertical is of itself important as a factor in that it directly affects convection currents.

[2] The general truth of the temperature-angle relation can be easily demonstrated, in a manner suggested by Prof. A. E. Douglas, University of Arizona, in the following experiment. Take a piece of heavy pine board, about 4 × 6 inches in size, and bore a shallow hole in the middle of one side. Connect this hole by a groove to one end. The groove is to carry the thermometer, whose bulb rests in the hole above mentioned. Fasten the board to uprights in such a manner as will permit it to swing on a horizontal axis, which, if continued, would reach the bulb of the thermometer. To the right hand upper corner attach a semicircular piece of white cardboard, ruled radially giving angles of 15°, 30°, and 45°. A nail is driven in the axis of the semicircle and allowed to project so that its shadow, falling on the cardboard, will give the angle of exposure desired. A thin sheet of copper is fastened over one side of the pine board, covering the thermometer. Finally, the wooden frame is enamelled white, and the copper sheet is painted black.

In order to check the observations made with the exposure pyrometer a black bulb thermometer, in vacuum, is used, and is read alternately with the other apparatus.

In practice, the test is made as follows. All instruments are allowed to come to shade temperature. Exposures to the sun of one minute each are made, and the instruments are allowed to return to the shade temperature, or close to it. The order of exposures, in the illustration given below, are as follows: 1, black bulb thermometer; 2, temperature at an angle of 90°; 3, black bulb; 4, temperature at angle of 60°; 5, black bulb; 6, temperature at angle of 30°; 7, black bulb; 8, temperature at angle of 90°; 9, black bulb.

The following data present results of a test on Feb. 6, 1915. The temperature rise in intervals of one minute and the time of each exposure are given.

Time	90°	60°	30°	90°	Black Bulb
10:44					5.1° C.
10:47	2.2° C.				
10:49		1.8° C.			
10:55					5.5° [1]
10:58			1.1° C.		
11:02					5.3°
11:06	2.3°				
11.08					5.3°

[1] The second reading of the black bulb thermometer was omitted.

At the Desert Laboratory there have been kept for several years three thermographic series giving the temperature of the soil. These series relate to three depths, namely, 15 cm., 30 cm., and 2.6 m.[3]

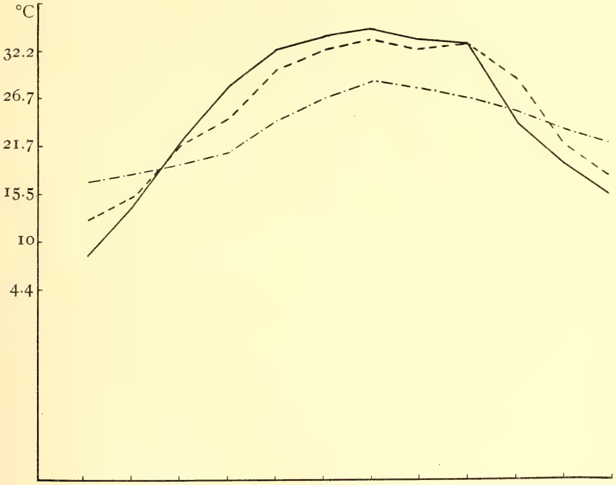

FIG. 2. Mean maxima soil temperatures, for different depths, at the Desert Laboratory, 1910. 15 cm. (———); 30 cm. (- - - - -); *ca.* 2.6 m. (—·—·—).

Since the soil temperatures obtained from the records have been discussed by several writers dealing with the botanical conditions of the region, only so much of them as will be necessary for illustrating the points brought out in this paper will be utilized. For this purpose the records covering a representative year, 1910, have been selected.

We will consider, in the first place, the temperature of the soil at a depth of 15 cm. In 1910 the mean maxima at this depth ran from

It will be seen that in the 24 minutes' duration of the experiment the energy, as measured by the black bulb thermometer, changed little. By assuming the readings of the 90° angle as a basis, we see that the relative values are as follows: 90°, 100 per cent.; 60°, 81.3 per cent.; 30°, 50 per cent., which are close to the theoretical percentages as given in figure 1.

[3] The thermographic records for the depth of 2.6 m. are of the constant temperature chamber, but it is assumed that they represent, sufficiently well for the present needs, the temperature of the soil at the depth given.

8.1° C., in January, to 34.0° C., in July, and the mean minima temperatures for the same months were 3.9° C. and 30.8° C., respectively.

The mean maxima for 1910, at a depth of 30 cm., range between 12.2° C., in January, and 33.0° C., in July. During June, July, August and September, the mean maxima at this depth did not fall under 32.2° C. The mean minima temperatures, of midwinter and midsummer, were 10.0° C., and 32.2° C.

At a depth of 2.6 m. the mean maxima temperatures, in 1910, ranged between 16.6° C., in January, and 27.2° C., in July.

When we compare the mean maxima temperatures for the three depths we notice that, from April to August, inclusive, the shallowest soil is also the warmest. We see, also, that in September the 30 cm. horizon has the highest mean temperature, and, finally, that during the winter months the highest temperature occurred in the deepest soil. The relation of the mean maxima temperatures for the three depths is given, month by month, in figure 2.

In the preceding paragraphs the actual soil temperatures have been considered, but another viewpoint, also instructive, can be had by integrating the temperatures of the records used above.

The results of such temperature summation, of all soil temperatures above 10° C., are presented graphically in figure 3. It will appear at once that the amount of heat in the soil, at a depth of 30 cm. beginning with April, was greater than the amount at the 15 cm. horizon, although the latter provided the higher maxima. The maximum amount of heat, at the 15–30 cm. depths, was received in June, while the maximum amount at a depth of 2.6 m. was not received until August. The sudden drop in temperature at the shallower depths seen in July is associated with the occurrence of the summer rains which began at that time. However, the penetration of the moisture did not serve wholly to arrest the upward temperature movement at the depth of 2.6 m. until the following month.

In figure 3 is given, also, as horizontal lines, integrations of temperatures averaging 20.0° C. and 30.0° C. They show, among other things, what portion of the entire year has soil temperatures averaging more than 20.0° C. and 30.0° C., and at what depths. In a general way, also, the two lines delimit the seasons of root growth, and to a degree shoot growth, also, of the shallowly rooted and the deeply rooted plants. Thus, vegetative activity can be seen in *Prosopis velutina* Wooton in March–April, while shoot growth in *Fouquieria* *splendens* Engelm. and in *Opuntia versicolor* Engelm, and active root growth also, as will appear below, does not occur until midsummer.

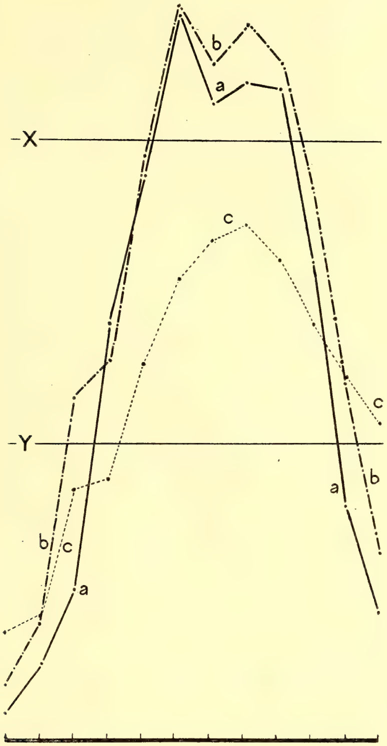

FIG. 3. Integration of monthly soil temperatures above 10° C., Desert Laboratory, 1910, at the following depths: 15 cm. (———); 30 cm. (—·—·—·); *ca.* 2.6 m. (- - - - -). The horizontal intersecting lines are integrations for an average temperature of 30° C. (*X*), and 20° C. (*Y*).

It should be interpolated here that root growth in the latter species, with shallowly placed roots, would take place in May–June were it not for the circumstance that this is the arid foresummer when root growth, and, broadly speaking shoot growth, also, of shallowly rooted plants does not occur.

Enough has probably been given concerning soil temperatures to show that roots which occupy different soil horizons in the same habitat are exposed to widely different temperatures, not only in winter, when there is little, or no root growth, but also in summer when the growth of roots is most active. We will now consider how differences in soil temperatures influence the root growth rate of species having unlike root-systems, namely, *Prosopis velutina*, and *Fouquieria splendens* and *Opuntia versicolor*.

Relation of Growth Rate in Roots to the Temperature of the Soil.

In order to observe the response of roots of typical desert perennials to variations in soil temperature three species were selected for study. These, as before mentioned, were *Prosopis velutina*, representing the deeply penetrating root type, and *Fouquieria splendens* and *Opuntia versicolor*, representing the type of root-system that lies near the surface of the soil. The growth rate of the roots was observed in several forms of experiments, and in very many experiments, which were carried out at the Desert Laboratory and at the Coastal Laboratory at Carmel, and, it can be premised that, in spite of the widely different environmental conditions in the midst of which the two laboratories are situated, the general results of the cultures and experiments were in all instances consistent.

A brief sketch of the leading types of experiments and cultures will be sufficient for the present purpose. In one form of experiments seedlings and relatively small plants were grown in tubes of different diameters and different lengths. These were either exposed to the temperatures of the air, or were kept in thermostats of which the temperature was known. In another type of experiments the species were grown in a wooden or metal box with a sloping glass side, and which, again, was either permitted to follow the prevailing air temperatures, or was heated artificially. In addition to these methods of handling the plants, seedlings and young plants were grown in the garden both at Tucson and at Carmel. In the present paper only the leading results of these numerous as well as diverse experiments will be given.

As regards the general results of growing young plants, and seedlings of *Fouquieria splendens*, and of *Opuntia versicolor* in tubes exposed to air temperatures, it can be stated, in brief, that root penetration of 1 m., or over, was obtained in each species in a single season. The air temperatures to which the tubes were exposed, and to which they fairly closely conformed, ran as high as 35.0° C.

In the box culture the roots of the same species as above used attained, within a period of 10 weeks, a depth of 47 cm. The temperature of the vertical soil column was fairly uniform and varied from 10.0° C. to about 45.0° C. The long root growth was obtained where the temperature was 30.0°–35.0° C.

At the time the box culture, just referred to, was running, seedlings of Fouquieria and of Prosopis were grown in the garden (of the Coastal Laboratory) where the surface soil varied in temperature from 10.0° C., to 22.0° C. Ten weeks after the seeds germinated it was learned that the roots of Fouquieria had attained a depth of 15–20 cm., and that the roots of Prosopis, on the other hand, were 32 cm., or over, in length.

Thus the tube, the box, and the garden cultures, to give no more, indicated that relatively deep penetration of the soil might be expected in the cases of Fouquieria and Opuntia if the soil is of a suitable temperature, but if this is not the case, the roots of these plants remain near the surface. On the other hand, the roots of Prosopis, in a relatively short time and in relatively cold soil, attained to a considerable depth, which, in fact, was approximately twice that of the roots of *Fouquieria splendens* of the same age and growing under like conditions.

In the other tube cultures referred to, the rate of root growth was observed day by day, or hour by hour, as the case might be, and temperature readings, either of the soil of the tubes, or of the chamber where the tubes were placed, were made at the same time. In the first of the more exact tube cultures the period of observation extended over a period exceeding 10 weeks, during which time a thermographic record was kept of the culture chamber, and, also, the soil temperature of the tubes was taken. The leading results of this series is shown graphically in figure 4. It will be seen, in brief, that the growth rate of the roots varies directly with the variation of the air temperature to which the tubes were exposed. Beyond this, however, it appears that, at parallel temperatures, the roots of Prosopis grew more rapidly than did those of the two other species. It is to be seen especially that

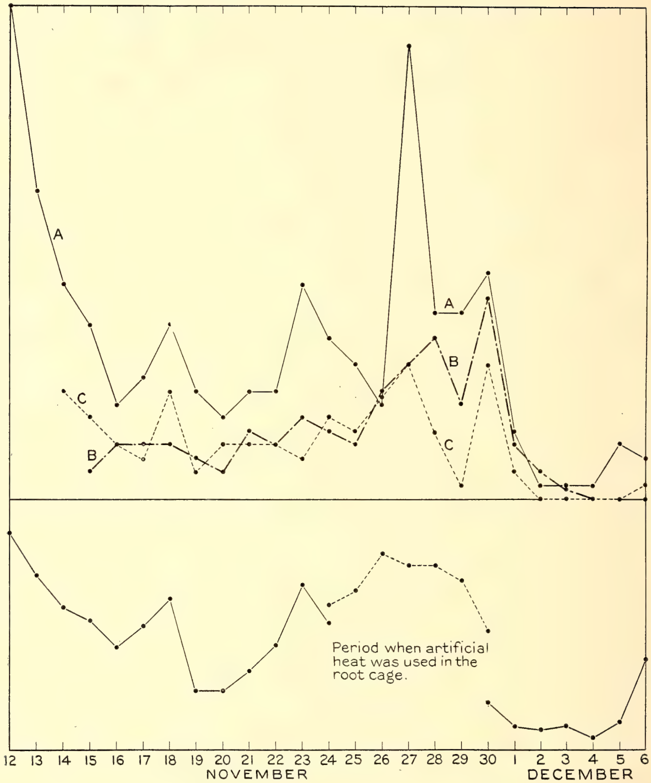

FIG. 4. Comparative daily growth rates of the roots of *Prosopis velutina* (*A*), *Opuntia versicolor* (*B*), and *Fouquieria splendens* (*C*), (much magnified), from November 12 to December 6, 1913. The lower curve gives the temperature summations above 10° C. in the root cage, and the detached curve is the summation when artificial heat was used in the cage. (From Year Book, Carnegie Institution, 1914.)

the growth rate of Prosopis roots at the lowest temperatures was relatively fast, and, also, that, at high temperatures, the roots of this

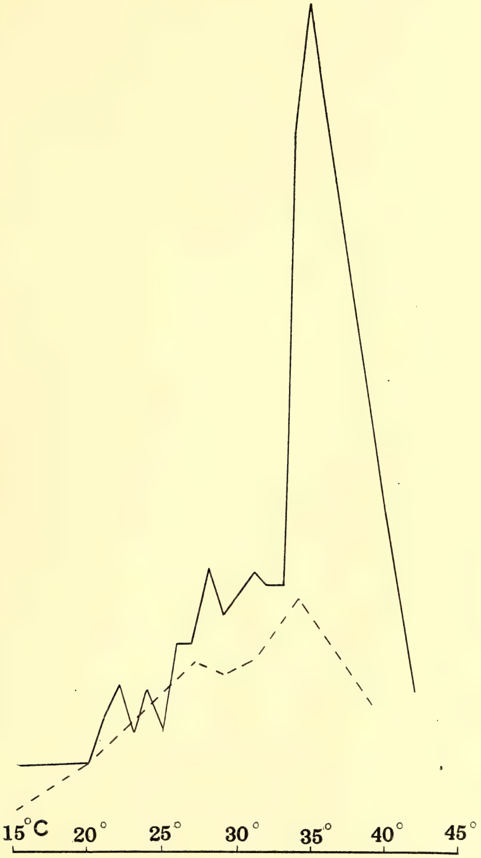

FIG. 5. Average hourly rate of growth of the roots of *Prosopis velutina* (———), and *Fouquieria splendens* (- - - - -) at the temperatures given (× *ca.* 100).

species grew at a more rapid rate than the roots of the other species. These points were subsequently verified in more precise cultures, as, also, they verify the previous observations already referred to.

The last series of experiments to be mentioned was that of tube cultures, also, in which special temperature control was used and in which thermostats of special design kept the tubes at temperatures between 20.0° C., or less, and 42.0° C., for as long periods as was desired. The readings of the root growth were usually made each hour at which time the temperature of the thermostat was also observed. In figure 5 appears the average rate of growth of the roots of *Fouquieria splendens* and *Opuntia versicolor*, taken from many readings, for the temperatures given. The growth rate is necessarily greatly magnified, but the comparative growth rates are consistent. To give an idea of the actual growth rates it can be said that the maximum average rate for Prosopis, reached at a temperature of 35.0° C. is 2.86 mm. per hour, and the maximum for Fouquieria was about 0.9 mm. per hour. The greatest growth rate observed in Prosopis roots was 3.0 mm. per hour. It will be seen that the rate of root growth falls away rapidly below 30.0° C. At 20.0° C. the rate for the roots of Fouquieria is very slow, practically ceasing at 15.0° C. In the case of Prosopis, on the other hand, a fairly active rate of root growth is maintained at 15.0° C., and, from other observations, it seems probable that growth continues at a temperature of 12.0° C. Thus the important fact is established that root growth occurs in Prosopis at a temperature so low as to be unfavorable for growth in Fouquieria, and *Opuntia versicolor*, also, although the root growth of the last species is not included in figure 5.

Root Development, Species Distribution, and the Reaction of Roots to the Soil Environment.

During the season of most active root growth, midsummer, there are three features in the root environment which are of especial importance, namely, the soil moisture, its temperature and aeration. It can be stated, in brief, that at this time, owing to the rains, the soil contains sufficient moisture for the developing plants, and, also, that the temperature of the soil, at the depths attained by roots, is suitable for their growth. Near the surface, in fact, as has already been shown, an optimum temperature for root growth obtains for a rather long period. Within limits, also, the moisture content of the soil increases, and the soil temperatures decrease with the depth beneath the surface. In addition to these features is the condition of the aeration of the soil. Air movements, of whatever kind, in adobe soil are profoundly influenced by the addition of water. That there is active movement of air in air-dry soil, and that the movement is strikingly affected by moisture can be easily demonstrated. For example, a tube 30 cm. long, filled closely with air-dry adobe, requires, under the conditions of the test, only 0.5 cm. water pressure to cause an active air current to pass through. On the other hand, it is extremely difficult to add so little water that the air flow is not quite stopped. And, of course, if sufficient water is added to puddle the soil, it is impossible to force air through. It happens, therefore, with the rains of summer and the consequent thorough wetting of the soil, that the movements of the soil atmosphere are greatly modified, and the ingress and egress of air cut down to a marked degree, or entirely stopped.

It is apparent, from what has already been given, that roots which lie close to the surface of the soil are subject to the influence of an environment which is quite different from that affecting the deeply placed roots. From the standpoint of experimentation it is best to assume, also, that it is owing to unlike responses to environmental conditions, that the characteristic differences in the mature root-systems is largely, if not wholly, due. Thus, so far as the Fouquieria-Opuntia species treated in the present paper are concerned, it appears that low soil temperature is a factor which limits downward root growth, while, on the other hand, this factor affects the root growth of Prosopis very little. From observations on the behavior of the roots of *Opuntia versicolor*, growing in tubes, it appears, also that root branching in this species is very closely related to good soil aeration. Whatever may be the factors that control root branching in Prosopis, on the contrary, it is probable that abundant aeration is not an important one. Thus, root growth and root branching in Fouquieria-Opuntia take place mainly near the surface of the ground where the aeration-temperature conditions are favorable, and the root-systems of these forms are superficial. As the lower temperature of the deeper soil does not inhibit root growth in Prosopis, and as the roots are not so restricted in the aeration relation, deep penetration results. And, thus, through unlike physiological response there results strikingly dissimilar root growth and development.

As regards the relation of root reaction and root type to species distribution, however, it must be admitted that it is a subject upon which up to the present very little work has been done. Enough has been accomplished on experimental and observational lines, however, to suggest the possible importance of the root relation in the distribution of species. The basis of the significance of the root factor in this connection lies in two general features, namely, in the root character itself and in the manner of response of roots to the soil environment. In the former case, especially in obligate deeply penetrating roots, the limiting factor appears to be only the depth of the soil. In such species, on the other hand, as have generalized roots and roots which are essentially shallowly placed, the condition is different. The limiting factors here relate to root response to such environmental soil factors as moisture, aeration and temperature.

From the position here taken, other distributional factors being equal, the species having the most plastic roots, and roots capable of the most catholic response to the soil environment, or both, should also be the most widely distributed, and conversely, species which are sharply limited in root growth by soil conditions, or whose roots are not plastic, should also have the most restricted distribution. So far as observations show the true condition, this conclusion is valid. From this it will appear at once that such factors as depth of soil, and soil moisture, aeration and temperature, are of the greatest importance. In addition there should be mentioned the exposure which, as already shown, greatly affects the temperature of the soil. It is of interest to note, also, that all of these, with the possible exception of the last named, are factors which are not commonly evaluated in a definition of a habitat and which do not immediately affect the shoots of plants.

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THE ANATOMY OF A HYBRID EQUISETUM[1]

Ruth Holden

The genus Equisetum has been the object of a considerable amount of study on the part of a great many botanists. As a rule, each has chosen certain features for especial investigation, with the result that each has adopted a different scheme of classification depending on what characters he himself deemed of most significance. Consequently, there is not a little divergence of opinion as to what features are of specific and what are of varietal importance. The confusion is increased by the large number of forms which differ from each other only in slight, though seemingly constant characters. For example, Mr. A. A. Eaton, who was the systematic authority on this genus in America, concluded that the true *Equisetum hiemale* L. is not found on this side of the water, but is replaced by *E. hiemale* var. *affine* (Engelm.) A. A. Eaton which differs in that the ridges of the stem are rounded instead of biangulate. As he expressed it (Fern Bulletin, v. II, p. 7) "the subgenus Hippochete is a multitude of races of which species are the centers of variation." Accordingly, he established a number of so-called varieties, which, as far as external features are concerned, bridge over by gradual steps all the gaps between different species. Thus, *E. arvense* L. grades into *E. littorale* Kühl through *E. arvense* var. *diffusum* and *E. littorale* var. *arvensiforme*. Duval Jouve (Histoire Naturelle des Equisetum de France), on the other hand, has done much to show that what have been described as separate varieties, or even separate species, are often merely fluctuations dependent on some environmental difference. For example, *E. fluviatile* L. was made a different species from *E. limosum* L. by Linnaeus, because the former has numerous branches, while the latter has none. Duval Jouve, however, claims that one grows in deep water and one in shallow, and that both species, with all the intermediate varieties, spring from the same rhizome. Milde (Monographia Equisetorum) goes further still, and considers that since *E. ramosissimum* Desf., *E. hiemale*, *E. trachyodon* A. Br., *E. variegatum* Schleich.,

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[1] Contributions from the Phanerogamic Laboratories of Harvard University, No. 65.

E. scirpoides Michx., *E. laevigatum* A. Br., and *E. robustum* A. Br. are all connected by transitional forms, they should be considered a single species. It may be possible to introduce some order into this chaos by a more careful anatomical study of these forms. As a possible first step in this direction, *E. variegatum* var. *Jesupi* A. A. Eaton has been chosen.

This variety was originally described by Eaton as "almost exactly intermediate between *E. variegatum* and *E. hiemale*," and the photographs of plate I show such to be the case. Figures 1, 3 and 5 represent respectively *E. variegatum*, *E. hiemale*, and *E. variegatum* var. *Jesupi;* figures 2, 4, and 6 the same at double the magnification. In size of stem and cone, the variety is approximately half way between *E. variegatum* and *E. hiemale*. According to Mr. Eaton, one of the best diagnostic characters is that of the sheath. In *E. variegatum*, it is loose, terminating above in persistent teeth (fig. 2). These teeth have a dark median groove, with a white border on each side, and end in deciduous awn like points. The sheath of *E. hiemale* on the other hand, is tight, with a black base and tip. The teeth are long, but articulated to the sheath and soon deciduous (fig. 4). The sheath of *E. variegatum* var. *Jesupi* is usually loose as in *E. variegatum;* like *E. hiemale* it has a black band, which in the second year reaches the base, while the upper part becomes ashy (fig. 6). The teeth are similar to those of *E. variegatum*, having the same dark median groove, and white border. Unlike *E. variegatum*, however, the awn points are usually persistent. Another fairly constant feature is the character of the ridges. This may be seen more easily in transverse sections, but it is indicated in the habit photographs. In *E. variegatum*, they are sharply biangulate, with a groove down the center of each. There is accordingly, a double row of siliceous tubercles to each ridge. Those of *E. hiemale* var. *affine*, on the other hand, are rounded, with a single row of tubercles. They may be clearly seen in figure 4. In *E. variegatum* var. *Jesupi*, the ridges are usually bluntly biangular, with rarely a carinal furrow. Like *E. variegatum*, there is usually a double row of tubercles (fig. 6).

With this brief account of the external appearance of these three Equiseta, we may turn to their internal structure. Figures 7, 9, and 11 represent respectively transverse sections of the internode of the aerial stems of *E. variegatum*, *E. variegatum* var. *Jesupi*, and *E. hiemale*. In some respects they are quite similar; all have a double endodermis continuous between the bundles; all have the sunken stomata characteristic of the subgenus Hippochaete; all have the stomata in two lines down the furrows, rather than scattered as in *E. littorale* and *E. limosum;* and all have the three sets of canals—central, vallecular and carinal. These sections corroborate the statements made above in regard to the outline of the stem. In *E. variegatum* (fig. 7) the ridges are sharply biangulate with a carinal furrow. Often in *E. variegatum* var. *Jesupi* (fig. 9) they are rounded, though not infrequently, bluntly angled. In *E. hiemale* (fig. 11) on the other hand, they are always rounded. These three photographs show also the relative size of the canals. The central and vallecular canals of *E. variegatum* have approximately the same diameter; in *E. variegatum* var. *Jesupi*, the central is considerably larger; and in *E. hiemale* this is still more the case.

Next to the distribution of the endodermis, the character of the bast fibers, with its consequent effect on the distribution of the green parenchyma, has been considered by anatomists as the most important diagnostic feature. In *E. variegatum*, there is a considerable mass of these fibres under both ridges and furrows, but it is usually heavier in the latter position. It extends inward to the border of the vallecular canal, thus cutting the green parenchyma at that point. This interruption of the palisade tissue under the furrows and its continuity under the ridges, seems to be invariable in *E. variegatum*, though Milde figures it as continuous around the whole stem. In *E. variegatum* var. *Jesupi* on the other hand, there is no such constancy. The carinal bast is usually more extensive than the vallecular, but, even so, the palisade tissue is not uncommonly continuous under it. Figure 8 shows a section where such is the case. The masses of vallecular bast are relatively small, but usually they reach the jacket of cells which surround the canals. This variability results in the green parenchyma being sometimes interrupted at only the furrows, sometimes at only the ridges, sometimes under both ridges and furrows (fig. 10), and rarely being continuous around the entire stem. Not infrequently, due to some irregularity, the carinal bast is pushed to one side of the ridge, and a similar displacement may occur to the vallecular. Toward the upper left of figure 9 instances of both these may be seen. Such a condition is at variance with Eaton's description. He saw the interruption of the green parenchyma by the carinal bast, but failed to see that by the vallecular. Inasmuch as the writer has had access to material from a number of different localities, some of which was identified by Eaton himself, it seems as though the error must be his. Figure 11 shows these structures in *E. hiemale.* According to Eaton, Duval Jouve, and Milde, the carinal bast in this species is the better developed, and interrupts the green parenchyma under the ridges, while it is continuous under the furrows. If figure 11 be examined with a hand lens, however, it will be found that while the masses of bast fibers at the ridges are larger than those at the furrows, there are invariably, as in *E. variegatum* var. *Jesupi*, cells extending inward from the latter which cut across the palisade tissue at that point, also. Figure 12 shows a small portion at a higher magnification, and there can be no doubt that such is the case. This section was cut from material grown in Toronto, Ont. On the other hand, plants grown in Cambridge, Mass., presented usually the conditions which have been described as typical for the genus—the parenchyma is continuous under the furrows.[2] The importance of these discrepancies is not necessarily great, Milde himself having found considerable variation throughout the genus, and especially in *E. hiemale.*

With this description of the aerial stems of the three Equiseta, it is clear that *E. variegatum* var. *Jesupi* occupies a position somewhat intermediate between the other two. Its bluntly biangled ridges are half way between the rounded ones of *E. hiemale* and the sharply angled ones of *E. variegatum;* while in the distribution of bast fibers and green parenchyma, it has the vallecular interruptions common to both the others, and the carinal interruptions of *E. hiemale.*

In the structure of the rhizome, there is also considerable divergence among the three. Figures 13, 15 and 17 represent respectively transverse sections of the internode of the rhizomes of *E. variegatum*, *E. variegatum* var. *Jesupi*, and *E. hiemale.* As in the aerial stems, the central canal of *E. hiemale* is much larger in proportion to the vallecular, than is the case with either of the others. The endodermis of *E. variegatum* has the same distribution in the underground as in the aerial stem—it is double, and continuous internally and externally. The endodermis of *E. hiemale*, on the other hand, is like that of *E. variegatum* in the aerial stem, but in the rootstock, it forms a separate sheath around each bundle as in *E. limosum*. In this respect, *E. variegatum* var. *Jesupi* is like *E. variegatum*, having a common internal and external sheath. Another feature of resemblance to *E. variegatum*, on the part of *E. variegatum* var. *Jesupi*, consists in the absence of socalled "nodal organs." These parenchymatous swellings, probably akin to lenticels, are as Dr. Jeffrey has pointed out (Structure, Development and Affinities of the genus Equisetum) present in *E. hiemale*, and lacking in *E. variegatum*. They seem to be absent also in *E. variegatum* var. *Jesupi*. Accordingly, as far as rhizome is concerned, *E. variegatum* var. *Jesupi* is considerably more like *E. variegatum* than *E. hiemale*.

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[2] Since writing the above, I have had an opportunity of investigating the stems of *E. hiemale* from Niagara Falls, Canada, and from the Cambridge, England, Botanical Gardens, and in all the specimens have found the green parenchyma continuous under the furrows.

Another fairly constant feature is the nature of the diaphragm, which occurs immediately above the node of both aerial and underground stems. Figure 14 shows a transverse section of *E. variegatum*, and figure 19, a longitudinal one. It is evident from these two photographs, that the upper surface of this diaphragm is covered with a layer of very thick walled, darkly staining cells. These are the tannin cells. They usually occupy the entire pith at this point, and also form large masses immediately inside the vascular bundles, extending upward for a considerable distance. Beneath them, are thinner walled, parenchymatous elements, which, toward the centre, show a tendency to become arranged in longitudinal rows. In *E. variegatum* var. *Jesupi*, the conditions are different, in that the tannin cells are considerably less in extent. Instead of occupying the entire pith, they appear in only a part of it (fig. 16). They are not equally developed in every node, so that, especially in the aerial stem, a longitudinal section may miss them altogether (fig. 21). On the other hand, the cells beneath are in distinct, longitudinal rows, forming a true periderm. As in other respects, *E. hiemale* goes one step beyond *E. variegatum* var. *Jesupi*. The tannin cells (fig. 18) occur only in isolated masses, and often, even in the rhizome, they fail to appear at all (fig. 23). The periderm, however, is very well marked, two or three layers of cells being lignified. Thus in the development of tannin cells and of nodal periderm, *E. variegatum* var. *Jesupi* is intermediate between *E. variegatum* and *E. hiemale*.

Finally, the cones of these three Equiseta were examined. Figure 20 represents a section of *E. variegatum*. In the centre is the sporophore, above and below which are the sporangia. The uniform size of the spores and their elaters coiled around them, are easily distinguished. The character of the wall may be best seen in the case of the two lower sporangia. It is one cell thick, and the cells themselves are covered with spiral thickenings. According to Duval Jouve, these spirals are characteristic of all species of Equisetum except *E. littorale* and *E. trachyodon.* Figure 24 represents a section of a cone of *E. hiemale,* including parts of four sporangia. Owing to defective technique, the spores are somewhat shrunken, but examination of fresh material, shows that all are spherical and all are uniform in size. Even in the section, the elaters appear clearly, and also the spirals in the walls. Figure 22 represents a single sporangium from a cone of *E. variegatum* var. *Jesupi.* Unfortunately the same shrinkage has taken place as in the cone of *E. hiemale,* but, despite that, it is evident that while some spores are normal in size, many are much smaller. The former always have elaters, while the latter often lack them. A further peculiarity is that the sporangium wall never has spirals. Through the kindness of the authorities at the Gray Herbarium of Harvard University, an opportunity was afforded to examine the cones of a number of specimens from eastern Canada and New England, including the type specimen from Royalton, Vermont, and in all the same mixture of normal and abnormal spores was found.

To sum up, *E. variegatum* var. *Jesupi* is like *E. variegatum* in the sheath with persistent teeth, in the location of the endodermis, in continuity of green parenchyma under the keels (rarely), and in absence of nodal organs in the rhizome. It is like *E. hiemale* in the black band of leaf sheath, and in the interruption of green parenchyma at both ridges and furrows (usually). It is intermediate between the two in height and diameter of stem, outline of ridges, relative size of vallecular and carinal canals, occurrence of tannin cells, and development of nodal periderm. Further, a large number of the spores are abortive, and the sporangium wall lacks spirals.

Before considering the significance of these facts, it may be apposite to recall the conditions in another member of the same genus, *E. littorale.* According to Milde, this species is like *E. arvense* in the constant presence of two rows of canals, in the presence of tubercles, and often in the form and color of the sheath; it is like *E. limosum* in the stomata, in the absence of a carinal furrow, in the teeth of the leaf sheath, and often in the form and color of the sheath. It is intermediate in the form of the green parenchyma which is sometimes like *E. arvense* and sometimes like *E. limosum;* in the cone axis which is neither hollow like the latter, nor entirely solid like the former; and in the central cavity which is half way between the two. Further, the spores are always partially abortive, and the sporangium wall lacks spirals. Milde, in company with most other authorities, has concluded from this evidence that *E. littorale* is a hybrid between *E. limosum* and *E. arvense.* Exactly the same arguments are applicable to *E. variegatum* var. *Jesupi,* which must be a hybrid between *E. variegatum* and *E. hiemale.* The presence of abortive spores, and absence of spirals in the walls of the sporangium seem to be reliable indications of hybridization, the present writer having found exactly the same conditions in a cross between *Betula pumila* and another birch.

It seems obvious from this evidence that *E. variegatum* var. *Jesupi* is clearly a hybrid, occupying an intermediate position between *E. hiemale* and *E. variegatum.* The question then arises whether it is the first filial generation of this cross. Before attempting to answer, there are various other facts to be considered. First, what is the relation of *E. variegatum* var. *Jesupi* to the European *E. trachyodon*? The latter is likewise considered by Alexander Braun to be intermediate between *E. hiemale* and *E. variegatum,* and indeed there are similar arguments for its hybrid nature as for that of *E. variegatum* var. *Jesupi.* Both have abortive spores, and both have a combination of the characters of *E. hiemale* and *E. variegatum,* but the combination is different. Each has a loose sheath with persistent teeth, but the bast of *E. trachyodon* is variable. According to Duval Jouve, the green parenchyma is interrupted under both ridges and furrows, while according to Milde, it is sometimes interrupted at only the ridges, and at others it is continuous around the whole stem. Moreover, while *E. variegatum* var. *Jesupi* has the endodermis characteristic of *E. varigatum, E. trachyodon* has that of *E. hiemale,* and further, *E. trachyodon* has the ridges of the stem biangulate, with a distinct carinal furrow. This last difference is discounted by the fact that the European *E. hiemale* has bluntly biangulate ridges, while ours has them rounded. Secondly, what relation do these varieties bear to *E. hiemale* var. *pumilum,* which Eaton considers intermediate between *E. hiemale* and *E. variegatum* var. *Jesupi,* or to *E. hiemale* var. *doelli,* which Milde considers intermediate between *E. hiemale* and *E. trachyodon?* A similar difficulty exists if *E. littorale* is a hybrid between *E. limosum* and *E. arvense:* where do *E. littorale* var. *arvensiforme* and *E. arvense* var. *diffusum* belong?

There are three possible answers: Either, as has been shown to be the case in birch crosses, the F_1 is very variable, so that some individuals are like one parent, and others like the other; or some, instead of being members of the first filial generation, are the result of long continued crossing and back crossing. The first explanation has one objection —why should *E. trachyodon* always be produced on the other side of the water, and *E. variegatum* var. *Jesupi* on this? Another difficulty is our ignorance of the way in which these infertile hybrids reproduce themselves. Are some of the spores capable of germination, or does the crossing have to take place anew for each individual? Although certain of the large spores are apparently normal, Duval Jouve, who had no difficulty in artificially cultivating other species of Equisetum, was unable to do so in the case of *E. littorale*. Furthermore, Milde has called attention to the fact that these supposed hybrids always grow in the vicinity of the parents.[3]

None of these points can be settled conclusively at the present time. The facts indicate that *E. variegatum* var. *Jesupi* is a hybrid between *E. variegatum* and *E. hiemale*, but until the cross is actually made under artificial conditions, the exact relation must remain uncertain. The genus Equisetum will be however a particularly favorable one for experimental work, since the various species are distinguished by a comparatively large number of clean cut and constant anatomical characters. Whether they are dependent on unit factors which are capable of independent segregation and recombination will be an extremely interesting and important subject for investigation.

SUMMARY.

1. *Equisetum variegatum* var. *Jesupi* is probably a hybrid between *E. variegatum* and *E. hiemale* for the following reasons: (1) It has some of the characters *E. variegatum*, some of those of *E. hiemale*, and some which are intermediate between the two. (2) A large number of its spores are always abortive.

2. Whether it represents the immediate offspring (F_1) of the cross, its relation to other varieties also intermediate between *E. variegatum* and *E. hiemale*, whether the cross is repeated for each individual, how far hybridization will explain the large number of transitional forms in

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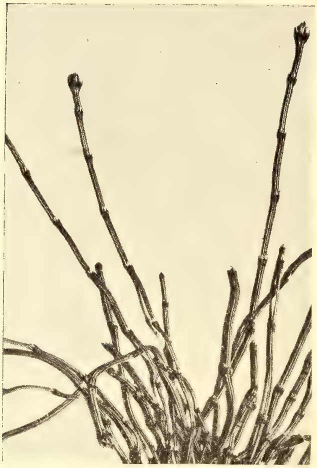
[3] A third explanation would be that instead of the result of crossing two species, three or even four may be implicated. Brainerd (Rhodora, March 1906) found indications of such complications in his work on violets, and cites one, *Viola* (*hirta* × *odorata*) × *V. collina*, from the Tyrol.

the genus Equisetum, are all points to be cleared up by further investigation.

In conclusion, I wish to express my best thanks to Professor Jeffrey for suggestions in regard to this work, and for material of *E. hiemale* and *E. variegatum* var. *Jesupi* from Toronto; and also to the authorities of the Gray Herbarium of Harvard University for material of *E. variegatum* and *E. variegatum* var. *Jesupi*, and for the loan of herbarium sheets from which the photographs of plate I were made.

EXPLANATION OF PLATES V–VIII

FIG. 1. Equisetum variegatum. × 1.
FIG. 2. Same. × 2.
FIG. 3. E. hiemale. × 1.
FIG. 4. Same. × 2.
FIG. 5. E. variegatum var. Jesupi. × 1.
FIG. 6. Same. × 2.
FIG. 7. E. variegatum, transverse section of aerial stem. × 60.
FIG. 8. E. variegatum var. Jesupi, transverse section of aerial stem. × 125.
FIG. 9. Same. × 30.
FIG. 10. Same. × 125.
FIG. 11. E. hiemale, transverse section of aerial stem. × 15.
FIG. 12. Same. × 125.
FIG. 13. E. variegatum, transverse section of internode of rhizome. × 60.
FIG. 14. Same, at node. × 60.
FIG. 15. E. variegatum, var. Jesupi, transverse section of internode of rhizome. × 60.
FIG. 16. Same, at node. × 60.
FIG. 17. E. hiemale, transverse section of internode of rhizome. × 15.
FIG. 18. Same, of node. × 15.
FIG. 19. E. variegatum, longitudinal section of rhizome. × 60.
FIG. 20. Same, section of sporophore and sporangia. × 60.
FIG. 21. E. variegatum var. Jesupi, longitudinal section of stem. × 40.
FIG. 22. Same, section of sporangium. × 250.
FIG. 23. E. hiemale, longitudinal section of rhizome. × 15.
FIG. 24. Same, section of sporangia. × 120.



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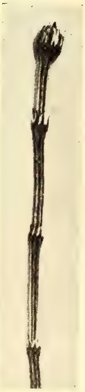

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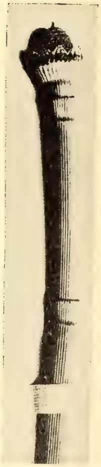

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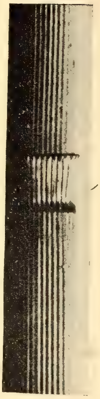

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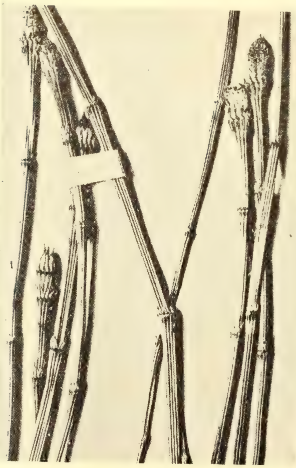

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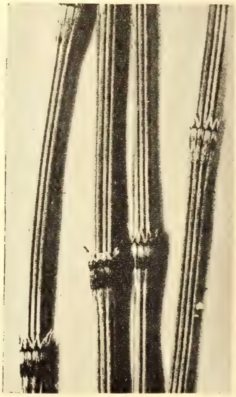

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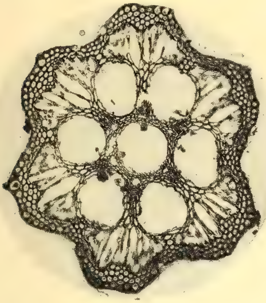
HOLDEN: ANATOMY OF A HYBRID EQUISETUM.



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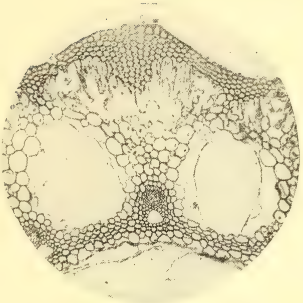

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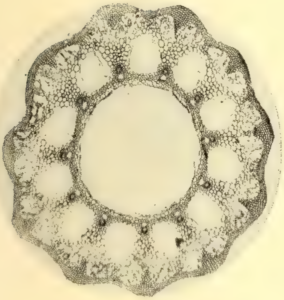

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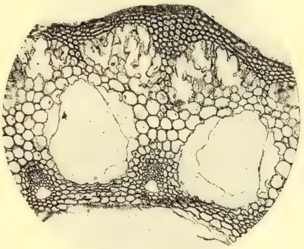

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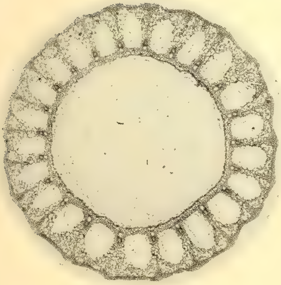

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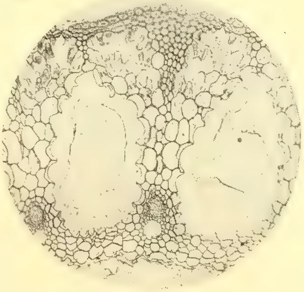

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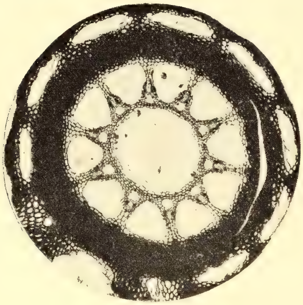
Holden: Anatomy of a Hybrid Equisetum.



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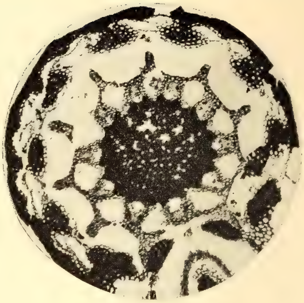

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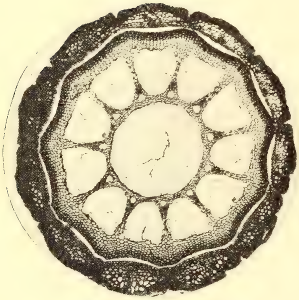

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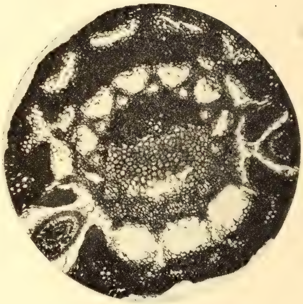

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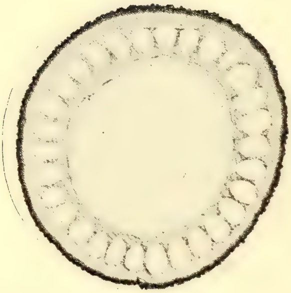

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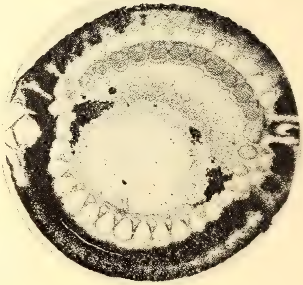

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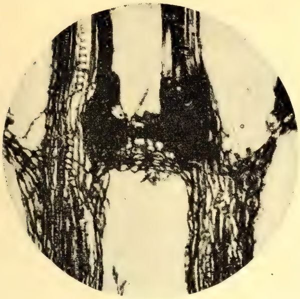
HOLDEN: ANATOMY OF A HYBRID EQUISETUM.



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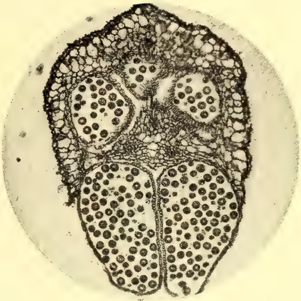

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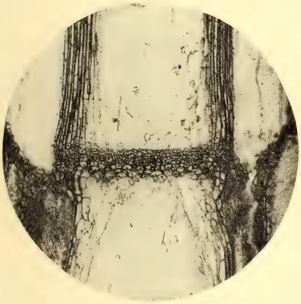

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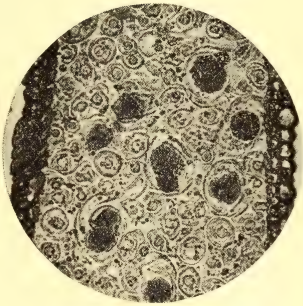

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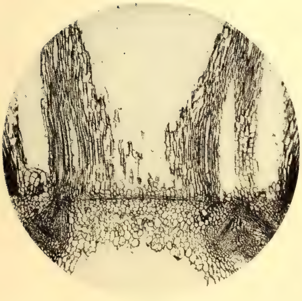

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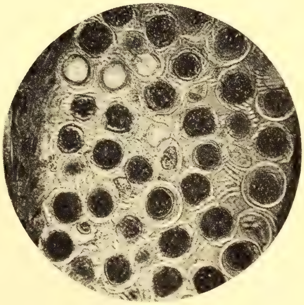

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HOLDEN: ANATOMY OF A HYBRID EQUISETUM.

SOME FEATURES IN THE ANATOMY OF THE MALVALES.[1]

C. C. Forsaith

In the study of the phylogeny of plants there are certain general principles upon which conclusions are based, among which the law of recapitulation is of the utmost importance. This law in its application to plant anatomy shows that primitive conditions are found in the seedling and in the first annual ring of growth. Furthermore, investigation has shown other primitive regions, viz., the root, the leaf petiole, and the reproductive axis. Experimental work has also demonstrated the value of traumatic regions. Studies in these directions have shown that the root is the most conservative, while the stem is the most progressive region of the plant, and consequently this latter organ loses its ancestral characters sooner than any other. It has further been shown that of these organs, the leaf petiole and reproductive axis are more progressive than the root, but not so much so as the stem, which makes them valuable as localities of transitional tendencies. Lastly, but by no means of the least importance, is the region of injury.

Many examples might be cited in illustration of these general principles taken from the anatomical features of the Conifers, an exceptionally favorable group for studies of this nature, owing to the abundance of fossil material of ancestral forms. One exemplification will serve. Pinus is considered to be the lowest genus of the Abietineae, a group in which resin canals appear normally in the woody cylinder, but in Abies, and other of the Abietineae, resin canals do not normally develop in the wood of the stem, but are found in the xylem of the root and other conservative organs, as well as in traumatic regions, thus illustrating the principles of retention and reversion. Since it has been somewhat generally admitted by competent investigators that the Abietae have been derived from the true pines, or Pineae, the natural inference is, that the conditions found in these conservative regions, just mentioned, may be taken as phylogenetic evidence of ancestral conditions (1). Still further illustrations of evolutionary tendencies in plants are found in Quercus, etc., by anatomical investigation and experiment. Studies of fossil ancestors of Quercus show that the uniseriate ray is primitive. A higher condition is seen where the rays are grouped in clusters to constitute the aggregate ray often found in relation to the leaf-trace. This condition is also present among others of the lower Cupuliferae, namely, Alnus, Carpinus, Corylus, and Betula. In this Angiospermous family, however, the conditions described are often found only in the first annual ring, or more persistently in the seedling, indicating that the aggregate ray is a relatively primitive condition, and consequently of phylogenetic importance (2).

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[1] Contributions from the Phanerogamic Laboratories of Harvard University, No. 66.

Another important anatomical feature is the distribution of wood parenchyma, in regard to which, a somewhat exhaustive investigation of many of the American species of the woody Dicotyledons has established, preliminarily, certain general conditions. The first is where the parenchyma is diffuse, that is, scattered throughout the woody portion of the stem; a second condition is vasicentric, and here the parenchyma is clustered around the vessels; the third mode is that where it is found only at the end of the annual ring, or terminal. That these respective conditions of distribution are of phylogenetic importance has been shown by investigation among the lower Angiosperms. The Casuarinaceae, Fagaceae, Betulaceae, Juglandaceae, Rosaceae, etc., show diffuse parenchyma; while studies of the higher forms, *i. e.*, the Oleaceae, Ulmaceae, Rhamnaceae, Parietales, Leguminosae, Compositae, etc., show vasicentric parenchyma. From these examples it is evident that the diffuse condition is the more primitive, since it is so characteristic of the lower orders, and that vasicentric is more advanced, since it is similarly characteristic of the higher orders. As terminal parenchyma occurs both in the higher and the lower orders of the Dicotyledons, it is evident it is not on the same footing as the other two methods of parenchyma distribution, and investigation has shown this to be the case. Miss Holden (3) obtained the following results in her studies of the Salicales: *Populus balsamifera* L., *nigra* L., *grandidentata* Michx. and *laurifolia* Ledeb. possess normally terminal parenchyma, but in the traumatic and conservative regions, vasicentric parenchyma was found. Similar conditions appeared in *Salix nigricans* Smith, *hookeriana* Barratt, *japonica* Thunb. and *daphnoidea* Vill.; but studies of the root, young stem, and wounded material, revealed also vasicentric parenchyma. In summing up the conditions found in the Salicales mentioned, the normal distribution was terminal, but vasicentric conditions were a feature of primitive regions. An application of the principles of comparative anatomy led to the conclusion that the species of Salix and Populus with terminal parenchyma were derived by reduction from those which possessed vasicentric storage cells, and were, therefore, less primitive species (3). It will be shown later that terminal parenchyma in the Angiosperms, although not in the Conifers, is the result of reduction.

The present article deals with conditions found in certain of the Malvales.

Beginning with the Tiliaceae, figure 1 shows a transverse section of *Tilia americana* L. From this it appears that the wood elements are all thin walled, and the numerous clustered vessels are diffuse. The wood parenchyma is present as thin walled cells extending as tangential bands between the rays, which become broadened in the phloëm region. In the upper portion of the figure alternate layers of hard and soft bast appear, of which the latter is composed of sieve tubes and storage cells. Figure 2 represents a tangential section of the same plant, showing one of the bands of parenchyma, the cells of which are indicated by thin-walled rectangular elements in the central portion of the illustration. A similar section through the first annual ring shows abundant diffuse parenchyma, which gradually becomes reduced to tangential bands in the later rings, shown in figure 1. Figure 2 also represents the numerous diffuse vessels with abundant, spirally arranged bordered pits and tertiary thickenings. However, no indication of fusion of the pits to mere slits, a condition common in the Malvaceae and Sterculiaceae, was found. The two types of rays are similiar to those which Bailey (op. cit.) mentions in his studies of Betula, Carpinus, Ostrya, and Corylus.

Figure 3 shows a transverse section of *Hibiscus tiliaceus* L. of the Malvaceae. Here the wood elements consist of bands of thick and thin-walled cells, alternately arranged between the two types of rays. The thin-walled portion is composed of clustered vessels and fibers, a condition very similar to that found in Tilia. This differs greatly from the thick-walled area, where the absence of vessels is conspicuous, and there is abundant diffuse parenchyma, which appears as thin-walled light and. dark cells. The dark appearance of certain cells represents the transverse wall of the wood parenchyma, in which simple pits may often be seen. Figure 4 illustrates a radial section of the same plant, on either side of which appears the thin-walled portion of the stem, consisting of vessels like those already described for Tilia. The central portion represents the thick-walled portion with its libriform fibers, and rectangular parenchyma cells with simple pits.

Figure 5 represents a transverse section of *Fremontia californica* Torr., which shows a striking resemblance to the thick-walled portion of Hibiscus, except for the presence of vessels. In this section parenchyma appears as thin-walled light cells, and darker ones where the cross wall is retained with its typical simple pitting. Figure 6 shows a radial section with thick-walled fibers, and diffuse parenchyma whose cross walls form rectangular cells.

Having presented the anatomical features of the three species, a discussion of their significance is desirable. The presence of diffuse parenchyma in Hibiscus and Fremontia, and the first annual ring of Tilia, with a reduction to tangential bands in the latter, shows that evidently the first two mentioned are more primitive since they do not show this characteristic reduction.

It may be advisable just to mention some of the characters found in the herbaceous forms of the Malvales. There is in all, naturally, a great reduction of the xylem tissue, which is brought about by the transition of fibers to wood parenchyma.

The New Zealand species of Aristotelia DC., shown in transverse section in figure 7, presents a departure from the characteristic diffuse type of parenchyma distribution, since storage cells occur only at the end of the annual ring, three terminal cells may be seen in the center of the figure, differentiated from the fibers by thin walls and simple pitting. Figure 8 represents a tangential section from the same material including the end of the annual ring. In this section a single terminal parenchyma cell is noticeable. The other structural features of the wood are not especially different from those found in Hibiscus and Fremontia.

Turning now to the Fagaceae, which show conditions very similar to those found in the species just described, figure 9 represents a transverse section of *Fagus grandifolia* Ehrh. The rays are of two types and are diffuse, as is also the parenchyma, which appears as thin-walled cells freely scattered throughout the wood. In radial section, figure 10, the parenchyma appears as vertical rows of thin-walled cells, which have been formed from the fibers by septation.

Conditions similar to those found in Aristotelia appear in a South

American species of Nothofagus, and are shown in figure 11, a transverse section. Wood parenchyma occurs only in a terminal position, and is frequently differentiated from the libriform fibers by the presence of crystals of calcium oxalate. A tangential section through the end of the annual ring, seen in figure 12, shows very clearly parenchyma cells, libriform fibers, and rays. An examination of the parenchyma shows, beside the simple pitting, the difference between ordinary parenchyma and crystal cells; the latter have been derived from the former by further subdivision.

Since the anatomical characters of Elaeocarpus correspond so closely to those found in Aristotelia, especially in respect to the distribution of wood parenchyma, it is evident that these features are the result of similar conditions of reduction.

Terminal parenchyma in the Angiosperms is apparently the result of reduction from ancestral forms in which either vasicentric or diffuse parenchyma was present. This has already been shown to be the case in the Salicales by Miss Holden, where those representatives which normally present only terminal parenchyma retain the vasicentric condition in traumatic and primitive regions, thus illustrating the principles of retention and reversion, respectively. Conditions very similar to those are to be seen in the Fagaceae, in which group, parenchyma occurs normally as cells scattered throughout the wood. Investigation of the aberrant South American representative, Nothofagus, shows only terminal parenchyma in the stem. This genus thus shows similarity, *mutatis mutandis*, to the Salicales investigated by Miss Holden (3).

From a consideration of the evolutionary tendencies in the two above mentioned families, it is evident that those which possess terminal parenchyma have suffered a reduction from more primitive ancestors which showed, in the one case, vasicentric, and, in the other, diffuse parenchyma, and consequently represent more advanced species.

Applying the above mentioned principles of Elaeocarpus and Aristotelia, two species which show terminal parenchyma, it is evident that they have been derived by reduction from more primitive ancestors; and, since the two examples cited show a reversion in recessive regions to the type of parenchyma distribution normally present in the less advanced members of the order, Elaeocarpus and Aristotelia may be considered as having suffered a reduction from ancestors, which possessed the diffuse type, and therefore, in this respect, represent a high development among the Malvales.

The Javanese species chosen from the Bombaceae, *Durio zibethinus* L., presents very interesting characters. Figure 13 shows a transverse section of a mature branch. Abundant diffuse parenchyma cells, including crystals, are characteristic for the species in addition to the normal type as found in the Tiliaceae. Figure 14, a higher magnification of the same area, shows better the average distribution of parenchyma, the cells of which are distinguished from the fibers by thin walls, simple pits, and crystals. Returning to figure 13, the central portion shows a departure from the normal structure in the presence of an aggregate ray with its included fibers. Figure 15, a radial section from the same material, shows, besides the rectangular parenchyma cells, those which have undergone secondary division to cubical cavities in which crystals are deposited. The aggregate structure of the ray shows even more clearly in a tangential section, figure 16, and in figure 17, a higher magnification of a single ray with its included fibers, which are in the process of transition to parenchyma, as is indicated by the septate condition. Figure 18 is similar to figure 16 except that a leaf-trace is included, thus showing the origin of the aggregate ray. The reason for the gradual transition to the diffuse type can be seen by a careful study of the tangential sections which show that all the included fibers are septate, an indication of transition to normal ray parenchyma without included fibrous elements.

The presence of aggregate rays in an order in which the diffuse type is normal is significant. Reference has already been made to the relative development of rays in which it was stated that the aggregate rays indicate a more primitive condition than those of a diffuse character, which points to the conclusion that, since Durio possesses this type of ray, it represents a more primitive condition than any of the other species of the Malvales described in this paper.

Still further proof of the presence of aggregate rays was presented by serial sections through several annual rings of growth in a stem which showed no indication of having been wounded, which, with the abundance of diffuse parenchyma, may be considered as indicative of primitive conditions. Consequently, so far as the anatomy of the stem is concerned, it is evident that Durio is the most primitive of the Malvales examined, since it still retains the aggregate ray as well as abundant diffuse parenchyma, which conditions are very similar to those already mentioned in reference to the Betulaceae; *i. e.*, *Betula populifolia* contains normally aggregate rays, while the more advanced species, *Betula alba*, shows them only in primitive localities.

Considering the Malvales as a whole from the morphological standpoint, it is evident that they represent a relatively low order among the Dicotyledons, and that the anatomical data appear to assign to them a position less high than that designated in the Syllabus der Pflanzenfamilien by Engler and Gilg, which places them between the Rhamnales and Parietales, both of which have vasicentric parenchyma, a condition very characteristic of the higher Dicotyledons. It may be stated here that in the investigations carried on in this laboratory, there has never been found a case where vasicentric parenchyma and aggregate rays were coexistent. Therefore, to place a group where aggregate rays occur and vasicentric parenchyma is characteristically absent, between two orders, which show diffuse rays and vasicentric parenchyma, appears to be unjustified.

There is a striking similarity in the morphological conditions found in the Malvales and the lower Dicotyledons in respect to the distribution of parenchyma which resembles that seen in Fagus, Betula, Alnus, Carpinus, and Corylus (all of which normally present diffuse wood parenchyma). In Fagus, however, there is a tendency toward a reduction to a terminal position, as mentioned above as an analogy to the conditions found in Aristotelia and Elaeocarpus. Further, this similarity is no more striking than is that of the ray structure, which, as in the above mentioned species, is normally of the diffuse type, with a tendency toward a reversion to the aggregate condition in Durio, which compares favorably with that found in Betula. Investigation has shown that in *Betula populifolia*, a recognized primitive species, aggregate rays occur normally, but in *Betula alba*, which is more advanced, such rays occur only in the first annual ring, or more persistently in the seedling.

From the study of the anatomical conditions found in the Malvales, it seems probable that they were descended from ancestors which normally possessed diffuse parenchyma and aggregate rays, which characters the tropical genus, Durio, still retains in the normal stem, and consequently appears from the anatomical standpoint to be the most primitive species examined. Without doubt from forms similar to this, the other genera were derived. The aggregate ray gave place to that of the diffuse type, as a result of the transformation of the included fibers to normal ray parenchyma, which, in the other species, takes place in the immediate vicinity of the leaf-trace.

During the gradual transition of the rays to those of a diffuse character, there has been, in some cases, a reduction of the wood parenchyma from the uniform diffuse condition, as seen in Durio and Fremontia, to the Hibiscus type, in which there are thin-walled areas in which the parenchyma is of infrequent occurrence. This process of reduction has reached a higher step in Tilia, where the parenchymatous wood elements occur only as tangential bands, with the exception of the first annual ring, where there is a reversion to the uniform diffuse condition. The climax of this process of reduction is reached in Aristotelia and Elaeocarpus, where it occurs only in a terminal position; and, applying the principles illustrated by Salix and Fagus, it is evident that these two genera represent the least primitive condition since they are the most reduced, and are, therefore, the most advanced, of all the Malvales mentioned in this paper.

Summary and Conclusions.

1. The primitive Dicotyledons had apparently diffuse parenchyma and aggregate rays.
2. All the Malvales examined, except Elaeocarpus and Aristotelia, show diffuse parenchyma in varying amounts.
3. Durio presents aggregate rays and diffuse parenchyma, and is therefore, to that extent, the most primitive of the Malvales examined.
4. Elaeocarpus and Aristotelia possess only terminal parenchyma, and therefore represent, in this respect, reduced species.
5. Applying general anatomical principles to the conditions cited above, it is evident that the Malvales came from ancestors possessing diffuse parenchyma and aggregate rays.

In conclusion, I wish to express my sincere thanks to Miss Frieda Cobb for material of Nothofagus; to the Director of the Harvard Botanical Garden for material of the herbaceous forms; and to Professor E. C. Jeffrey for his helpful advice and aid in securing the photomicrographs accompanying this article.

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EXPLANATION OF PLATES IX–XI

FIG. 1. Tilia americana L.: transverse section of normal wood, showing diffuse parenchyma and thin-walled elements. × 200.

FIG. 2. Same: tangential section, showing diffuse parenchyma and rays. × 200.

FIG. 3. Hibiscus tiliaceus L.: transverse section, showing diffuse parenchyma and thick and thin-walled elements. × 200.

FIG. 4. Same: radial section, showing diffuse parenchyma. × 200.

FIG. 5. Fremontia californica Torr.: transverse section, showing diffuse parenchyma and thick-walled wood elements. × 200.

FIG. 6. Same: radial section, showing diffuse parenchyma. × 200.

FIG. 7. Aristotelia DC.: transverse section, showing terminal parenchyma. × 200.

FIG. 8. Same: tangential section through the end of the annual ring, showing terminal parenchyma. × 200.

FIG. 9. Fagus grandifolia Ehrh.: transverse section, showing diffuse parenchyma. × 200.

FIG. 10. Same: radial section, showing diffuse parenchyma. × 200.

FIG. 11. Nothofagus: transverse section, showing terminal parenchyma and crystal cells. × 350.

FIG. 12. Same: tangential section through the end of the annual ring, showing terminal parenchyma and crystal cells. × 200.

FIG. 13. Durio zibethinus L.: transverse section, showing diffuse parenchyma and aggregate rays. × 150.

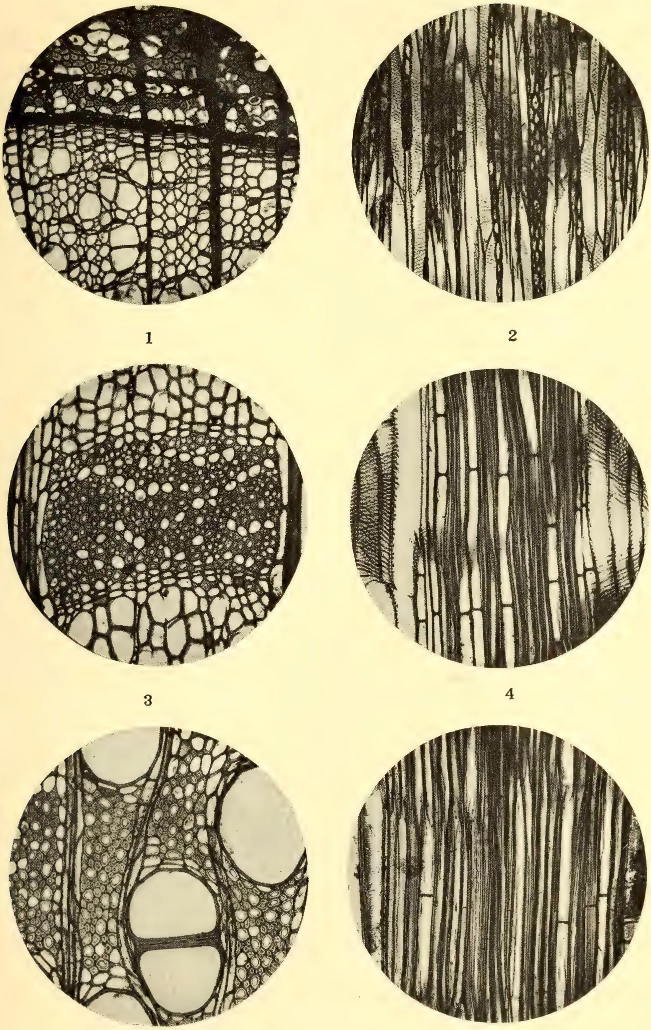
FIG. 14. Same: showing diffuse parenchyma and crystal cells. × 400.

FIG. 15. Same: radial section, showing diffuse parenchyma and crystal cells. × 250.

FIG. 16. Same: tangential section, showing aggregate rays and diffuse parenchyma. × 45.

FIG. 17. Same: showing aggregate ray with included septate fibers. × 120.

FIG. 18. Same: showing aggregate rays and leaf-trace. × 18.



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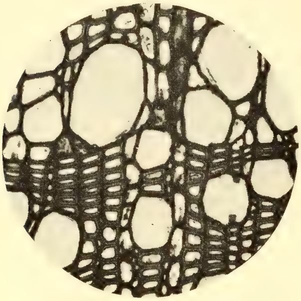
FORSAITH: ANATOMY OF THE MALVALES.



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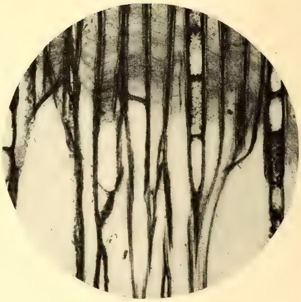

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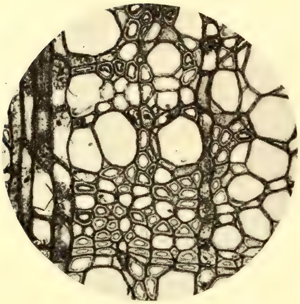

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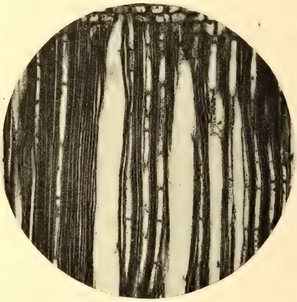

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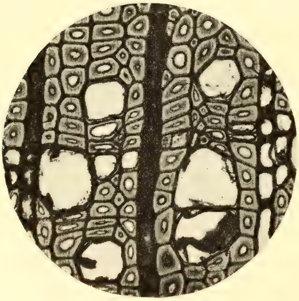

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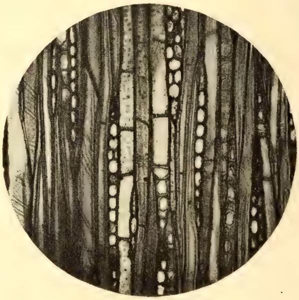

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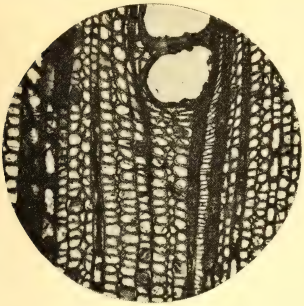
Forsaith: Anatomy of the Malvales.



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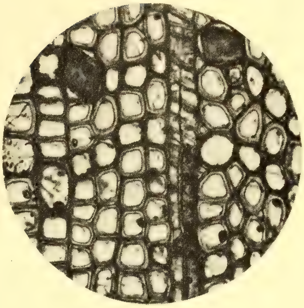

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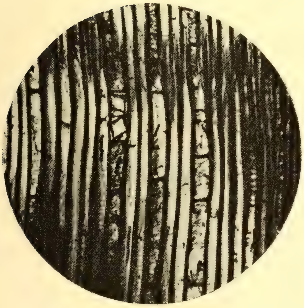

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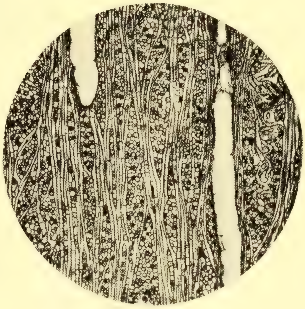

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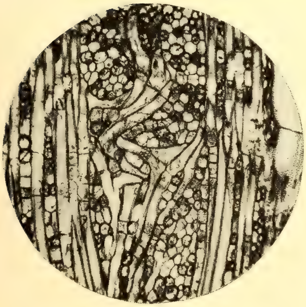

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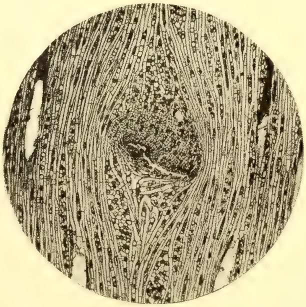

18

FORSAITH: ANATOMY OF THE MALVALES.

THE ABSORPTION OF IONS BY LIVING AND DEAD ROOTS

H. V. Johnson

It is a well-known fact that roots often produce an acid or an alkaline reaction in solutions of neutral salts.[1] This might be ascribed to the excretion of acid or alkali by the root or it might be explained on the ground that the root takes up from the solution more kations than anions (making the solution acid) or an excess of anions (making the solution alkaline).

If the root excretes acid or alkali we should expect to find these substances given off in distilled water. A number of investigators have reported that distilled water in which roots are placed frequently becomes acid.[2]

The writer has confirmed this and has found that after thorough boiling (to drive off CO_2) the reaction may still be acid to litmus. In these experiments the writer employed roots of corn and took care not to allow the seeds to come into contact with the solution.

The execretion of alkali by roots may be considered doubtful, although the excretion of alkali by algae has been reported.

The amount of acidity and alkalinity produced by roots in solutions of neutral salts seems in many cases too great to be accounted for by excretion and it is presumably due to the absorption by the roots of anions or of kations. It is evident that if this is the usual method of absorption it deserves careful investigation. Little attention has so far been paid to the mechanism of this process. It is evident that kations can not be absorbed without anions (or vice versa) for this process would soon be stopped by the resulting electric stresses. It has therefore been suggested that an exchange of ions occurs, the plant giving up a kation for each kation it absorbs, and vice versa. It has also been pointed out that the same thing may occur in adsorption by various colloids.[3]

---

[1] Cf. J. F. Breazeale and J. A. LeClerc, U. S. Dept. Agric. Bur. Chem. Bull. **149**; E. J. Russell, Soil Conditions and Plant Growth, p. 119; R. Meurer, Prings. Jahrb. Wiss. Bot. **46**: 503. 1909; and the literature cited in these articles.

[2] Cf. F. Czapek, Biochemie der Pflanzen **2**: 873. 1905; J. Stocklasa und A. Ernst, Prings. Jahrb. Wiss. Bot. **46**: 73. 1908; R. Meurer, ibid. **46**: 503. 1909; T. Pfeiffer und E. Blanck, Landw. Versuchsst. **77**: 217. 1912, and the literature cited in these articles.

It is of course possible that the root gives off hydrogen ions only when it absorbs a corresponding number of other kations. In this case excretion of acid by the root would take place only in solutions of salts. In the same way hydroxyl ions might be exchanged by the root for other anions.

It is evident however that all the facts can be accounted for in another way. The hydrolytic dissociation of neutral salts produces both acid and alkali and, as has been pointed out by Osterhout,[4] if one of these is absorbed by the plant more rapidly than the other the solution must become acid or alkaline.

It is evident that an exchange of ions may occur without altering the reaction of the solution. In such cases the exchange could be detected by an analysis of the solution, before and after the roots have acted upon it. This method has been employed by Meurer[5] and others.

One point which seems to have been lost sight of is that in experiments of this sort the unequal absorption of anions and kations may be due to the dead rather than to the living cells of the root. Thus for example in the extensive experiments of Meurer on beets and carrots the outer layers of the root were cut away by means of a knife. This killed the cells for some distance below the surface so that the solution came into contact with a large mass of dead cells before it penetrated to the living tissue.

It is to be expected that this would affect the results and the writer's experiments show that this is the case. For example the writer has found that live beets removed, from a solution of $CaCl_2$, 41.31 per cent. of the Ca present and 43.74 per cent of Cl, while dead beets removed of 44 per cent of Ca and only 26.25 per cent of Cl (Table I, averages). Carrots gave a similar result, the live plants removing 9.65 per cent of Ca and 7.8 per cent of Cl while the dead removed 53.87 per cent of Ca and 28.57 per cent of Cl (Table I, averages).

---

[3] von Bemmelen, Zeitschr. Anorgan. Chem. **23**: 321. 1900; Höber, Physikalische Chemie der Zelle und der Gewebe, S. 239, 1914.

[4] Science n. ser. **36**: 571. 1912.

[5] Meurer, R. Prings. Jahrb. Wiss. Bot. **46**: 503. 1909.

TABLE I

Beet, Carrot and Turnip in Solution of $CaCl_2$

	Duration of Experiment in Days	Gram Ionic Conc. of Ca		Gram Ionic Conc. of Cl		Per Cent Ca Removed	Per Cent Cl Removed
		At Start	At End	At Start	At End		
Beet	8	.0222	.0188	.0422	.0345	15.3	18.2
Alive	17	.0222	.0170	.0422	.0319	23.4	24.4
Alive	8	.0222	.0178	.0422	.0340	19.8	19.4
Alive	39	.0222	.0088	.0422	.0170	60.4	59.7
Alive	17	.0206	.0130	.039	.0228	36.78	41.47
Alive	17	.0206	.0094	.039	.0147	54.53	62.31
Alive	17	.0206	.0104	.039	.0191	49.34	50.96
Alive	26	.0206	.0087	.039	.0156	57.57	60.00
Alive	14	.0206	.0094	.039	.0128	54.53	67.18
					Average	41.31	43.74
Dead (chloroform)	3	.0222	.0109	.0422	.0270	50.9	36.0
Dead (formalin)	9	.0221	.0139	.0420	.03505	37.1	16.5
					Average	44.0	26.25
Carrot							
Alive	9	.0222	.0198	.0422	.0382	10.8	9.5
Alive	11	.0222	.0203	.0422	.0396	8.5	6.1
					Average	9.65	7.8
Dead (chloroform)	3	.0222	.0123	.0422	.0333	44.6	21.1
Dead (chloroform)	4	.0222	.0116	.0422	.0312	47.7	26.1
Dead (formalin)	9	.0221	.0131	.0420	.0341	40.7	18.8
Dead (formalin)	17	.0206	.00704	.039	.02235	65.56	42.56
Dead (formalin)	17	.0206	.00602	.039	.0256	70.78	34.31
					Average	53.87	28.57
Turnip							
Dead (chloroform)	2	.0221	.0169	.0420	.0350	23.5	16.6

In the writer's experiments uninjured beets or carrots were placed in the solution (the top projecting out of the liquid) and allowed to remain for the period indicated. The concentration of ions was determined before and after the roots were placed in the solution. The Ca was determined by precipitating with ammonium oxalate, igniting and weighing as CaO; the Cl was titrated with 0.1 M $AgNO_3$, using potassium chromate as indicator. The amount of experimental error is shown by comparing the determinations of the concentrations of the two ions at the beginning of the experiment as given in the table (if there were no experimental error the gram ionic concentration of Cl would be exactly twice that of Ca).

In subsequent experiments corn was germinated in tap water. When the roots had reached a length of one or two inches and were in active growth, so that no dead cells were observable, they were placed

TABLE II

Roots of Corn in Solution of $CaCl_2$

Lot	Roots of White Field Corn	Duration of Experiment in Days	Gram Ionic Conc. of Ca		Gram Ionic Conc. of Cl.		Per Cent Ca Removed	Per Cent Cl Removed
			At Start	At End	At Start	At End		
1	Alive	15	.0221	.0192	.0420	.01823	58.4	56.6
2	Alive	14	.0226	.0117	.0429	.0231	48.2	46.1
3	Alive	14	.0226	.0135	.0429	.02536	40.3	40.9
4	Alive	7	.0226	.0107	.0429	.0220	52.6	48.7
5	Alive	7	.0226	.0079	.0429	.01693	65.0	60.5
						Average	52.9	50.56
6	Dead (killed by boiling then in $CaCl_2$ and formalin)	5	.0221	.0133	.0420	.0254	39.8	39.5
7	Dead (from lot 1, in $CaCl_2$ and formalin)	4	.0221	.0186	.0420	.03496	15.8	16.8
8	Dead (from lot 2, in $CaCl_2$ and formalin)	3	.0226	.0221	.0429	.04053	2.2	5.5
9	Dead (from lot 3, in $CaCl_2$ and formalin)	3	.0226	.0186	.0429	.03313	17.7	22.8
10	Dead (in tap water; then in $CaCl_2$ and formalin)	3	.0226	.0175	.0429	.03386	22.5	21.1
11	Dead (in tap water; then in $CaCl_2$ and formalin)	3	.0226	.0182	.0429	.13513	19.5	18.1
12	Dead (from lot 4; in $CaCl_2$ and formalin)	2	.0226	.0192	.0429	.03926	15.0	8.5
13	Dead (from lot 5; in $CaCl_2$ and formalin)	2	.0226	.0210	.0429	.04003	7.1	6.7
						Average	17.45	17.37
	Roots of Sweet Corn							
14	Dead (in tap water; then in $CaCl_2$ and chloroform)	5	.0206	.00597	.039	.0136	71.0	65.13
15	Dead (in tap water; then in $CaCl_2$ and chloroform)	5	.0206	.00606	.039	.0135	70.56	65.38
16	Dead (in tap water; then in $CaCl_2$ and chloroform)	5	.0206	.00784	.039	.0168	61.66	56.92
17	Dead (in tap water; then in $CaCl_2$ and chloroform)	5	.0206	.00918	.039	.0189	55.41	51.54
						Average	64.66	57.59

in $CaCl_2$. (The seeds were not allowed to come into contact with $CaCl_2$.) In some cases sufficient chloroform or formalin was added to the $CaCl_2$ to kill the roots and the results were then compared with those obtained with living roots. In experiments with sweet corn the dead roots took up somewhat more Ca than Cl but this was not true of white field corn (Table II, averages). A single experiment on dead turnips gave a result similar to that obtained with sweet corn (Table I).

It is therefore evident that in some cases the presence of dead cells has a very marked influence on the results. It should be borne in mind that even when all the root cells are alive at the beginning of the experiment some of them may be killed by the solution while the experiment is going on. For this reason the writer employed $CaCl_2$ in his experiments, as calcium salts are in general less toxic than the other common salts of the soil. In long experiments it seems advisable to employ, whenever possible, balanced (or non-toxic) solutions for this purpose.

Laboratory of Plant Physiology,
Harvard University.

[The Journal for April (2: 157–198) was issued 13 May 1915.]

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THE EXCHANGE OF IONS BETWEEN THE ROOTS OF LUPINUS ALBUS AND CULTURE SOLUTIONS CONTAINING ONE NUTRIENT SALT[1]

RODNEY H. TRUE AND HARLEY HARRIS BARTLETT

Former work by one of us[2] has shown that the roots of *Lupinus albus* excrete electrolytes when grown in distilled water. From river water, on the contrary, the roots actively absorb electrolytes. There is a decided difference in root growth in the two cases. As compared with river water, the distilled water causes a decided inhibition of growth which is doubtless correlated with the loss of necessary salts from the roots to the distilled water. When quantities of certain calcium or sodium salts equal osmotically to the total salt content of Potomac River water are added to distilled water the loss of ions from the roots is found to be checked by the presence of calcium salts, but not by the presence of the sodium salts. Growth in the calcium solutions is found to equal that in river water checks; that in NaCl solutions being only slightly and temporarily better than in distilled water. The writers[3] have made a study of the concentration relations of dilute solutions of calcium and magnesium nitrates to the roots of *Pisum sativum*, and have found that for each of these salts or for a mixture of the two, there is a fairly definite concentration at which excretion

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[1] Published by permission of the Secretary of Agriculture.

[2] True, Rodney H., The Harmful Action of Distilled Water. Amer. Journ. Bot. 1: 255–274. 1914.

[3] True, R. H., and Bartlett, H. H., Absorption and Excretion of Salts by Roots, as Influenced by Concentration and Composition of Culture Solutions. I. Concentration Relations of Dilute Solutions of Calcium and Magnesium Nitrates to Pea Roots. Bulletin 231, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C., 1912.

[The Journal for May (**2**: 199–254) was issued 16 June 1915.]

of salts by the roots is equalled by absorption. This equilibrium concentration is lower for calcium nitrate than for magnesium nitrate, and lower for an equimolecular mixture of the two than for either alone. In the latter case it is about a hundred-thousandth molecular. If the initial concentration of the culture solution is less than the equilibrium concentration, excretion from the roots predominates over absorption and brings about a net rise in the concentration. Conversely, if the concentration of the solution is initially above the equilibrium point, the roots absorb more salts than they excrete and the concentration becomes less. The results with peas showed, furthermore, that with a culture solution of appropriate concentration and composition, absorption may take place until the solution becomes almost as free of electrolytes as distilled water. In such a case, excretion is obviously a negligible factor, and the net change in concentration of the solution must be ascribed to absorption alone. Absorption was found to be greatest in solutions containing calcium and magnesium nitrates in equimolecular proportions, but root growth was found to be equally good in any solution which did not contain less than one molecule of the calcium salt to nine of magnesium. In the more dilute solutions an even lower ratio of calcium to magnesium, 1 : 99, was found to completely overcome the deleterious effect exerted by magnesium alone. In other words absorption and growth appear to be more or less independent phenomena.

The present paper extends the same line of investigation to other salts and to a different plant. *Lupinus albus* L. has been studied in a considerable range of concentrations of $Ca(NO_3)_2$, $CaSO_4$, $Mg(NO_3)_2$, $MgSO_4$, KNO_3, K_2SO_4, KH_2PO_4, KCl and NaCl. Future articles will deal with the effect of various mixtures of these salts in pairs and in threes on the same plant.

METHODS

The methods used were in general those described in our former article on peas (l. c.). Carefully selected seeds of *Lupinus albus* were germinated in moist sphagnum and used when the primary root was about 50 mm. long. Four roots were used in each 500 c.c. culture. Readings were made daily until it became apparent from the appearance of the plants that they were approaching exhaustion. This stage was marked by the withering of the foliage leaves, the partial dying of the edges of the cotyledons, the frequent failure of the hypocotyls and by the loss of electrolytes from the roots.

Jena glass beakers were chosen as containers, after preliminary experiments had shown that any difference between Jena glass and opaque quartz or paraffined glass due to the solubility of the former was much less than the experimental error from other causes. For example the diffusion of carbon dioxide from freshly distilled water is often sufficient to increase the electrical resistance of water left standing in any one of these three types of containers, thus offsetting any change in the opposite direction due to the solubility of the container. In fact, a common method of improving distilled water for use in conductivity determinations is to pass through it a stream of air, free of carbon dioxide. The loss of carbon dioxide from distilled water left standing in contact with the air is sufficient to lower its conductivity very appreciably. The water used for distillation was moderately "hard," *i. e.*, it contained bicarbonates which liberated carbon dioxide during the process of distillation. The fresh distilled water therefore contained more carbon dioxide than it did later after standing in contact with the air. It is self evident that water which is to be used for culture solutions should not be freed from carbon dioxide, in case it is wished to study the excretion or absorption of other electrolytes than carbonic acid from the roots. Since there must be a considerable lag in the attainment of CO_2 equilibrium between air and a solution to which CO_2 is supplied continuously, it follows that the electrolytic method shows, as the first effect of placing roots in distilled water, an increase in conductivity due to the excretion of CO_2. It follows that if CO_2 constantly diffuses into the air from the solution, increase in conductivity on account of excretion of CO_2 from the roots must reach a maximum and thereafter remain reasonably constant as long as the roots continue to supply CO_2. The maximum conductance due to CO_2 will be reached when the rate of diffusion of CO_2 from the solution into the air equals the rate at which CO_2 is supplied by the roots. The error in the conductance of a solution due to CO_2 can not be less than the conductance of water in equilibrium with air containing 3 parts in 10,000 of CO_2. This lower limit of error is about equivalent to the conductance of half-millionth molecular NaCl. The upper limit of error can be approximately estimated as equivalent to the conductance of 16 millionths molecular NaCl. This estimate is based upon the measurement of absorption from solutions which show almost perfect absorption of the salt content by the roots. In reality the error is not as great as this figure, for probably no culture solution is ever so completely depleted of ions that none remain except those resulting from the dissociation of carbonic acid.

Stock solutions (from which the culture solutions were made by dilution with distilled water) were accurately prepared from Merck's best "blue label" reagents.

In our experiments a range of concentrations was used comparable to the range from distilled water to the less concentrated soil solutions. The more concentrated culture solutions, for example, contained less mineral matter than the water from the Potomac River, but more than the water of the Great Dismal Swamp of Virginia. In the case of the simple salts the range of concentration in the culture solutions was greater than the range of the same single constituent among average soil solutions. In other words, we made an endeavor to study the reaction of the plant toward solutions similar in concentration to those which it might encounter under natural conditions.

It will be seen from the accounts of the particular experiments that the cultures were not kept at constant temperature. In order to avoid the deleterious effect of illuminating gas on the cultures, they were set up in a dark cabinet at the greenhouse. Although danger from gas leakage was thus avoided, it was found impossible, with the means at our disposal, to eliminate a considerable temperature fluctuation. The temperature of each culture was read daily when the conductivity was determined. It will be seen that the temperature curves, which are plotted parallel with the concentration curves for each experiment, show no consistent influence of temperature upon absorption or excretion. As a matter of fact, the most perfectly executed experiments, such as those charted in figs. 1 and 2, show no temperature effect whatever. Possibly this interesting fact may be correlated with certain observations made by Eckerson.[4] This investigator has found, for example, that the primary roots of Phaseolus show no increase in permeability from 13° to 25° C. Similar phenomena were observed with other plants.

In our experiments concentrations were determined in the following manner. On the day that a series of cultures was prepared, the conductivity of each solution was determined at room temperature before the roots were placed in it. As long as the experiment lasted, the conductivity was read daily, each time at room temperature. It was impracticable to bring the culture solutions to constant temperature before reading them, however desirable such a procedure might have been. It is well known that the temperature coefficients of conductivity for very dilute salt solutions, in which carbon dioxide exists in considerable concentration, are not the same as the coefficients for concentrated, relatively uncontaminated solutions. It was within the limits of accuracy of our work to use for all the dilute salt solutions the coefficient 0.20. All conductivities are referred to 18° C. Since in conductivity work with solutions of even $M/50$ concentration the impurity of the water may require a correction of 0.1 per cent., it is quite obvious that in some of our more dilute culture solutions the concentration increment between contiguous cultures was less than the initial concentration of impurities in the water. It was found, however, that the effect on conductivity of adding small increments of salt to distilled water was additive. Consequently we have assumed that the changes in conductivity of the solutions during the period of the experiment were likewise due to addition or subtraction of electrolytes. In the presentation of our results we have translated the changes brought about by the roots in the conductivity of the culture solutions into terms of change in concentration of the particular salt used in each experiment. All concentrations were determined with reference to the conductivities of the culture solutions as originally prepared, before the roots had been placed in them. As already remarked, at least part of the initial rise in concentration of most culture solutions after the roots are placed in them may be ascribed to carbon dioxide. Presumably the absorption of salt from all solutions was greater than the figures show, by the amount of this initial rise in the CO_2 concentration. As we have already pointed out, the error from this source can never have been as great as would correspond to a change in concentration of $14M \times 10^{-6}$, in the case of a salt like NaCl.

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[4] Eckerson, Sophia, Thermotropism of Roots. Bot. Gaz. **58**: 254–263. 1914.

In connection with the fact that not only absorption but also excretion was measured in terms of the original salt used in spite of the fact that we know nothing in regard to what the roots may actually have excreted, it may be stated that most of the commoner electrolytes have nearly enough the same equivalent conductance so that little error would enter into our results on this account. At the close of each experiment the following measurements were taken for each plant: The length of the primary root, the length of several of the longer lateral roots, the length of the hypocotyl, and the length of the first internode above the cotyledons. It was found that only a very loose relation existed between the length of the first internode and the length of the hypocotyl on the one hand, and either the development of the root system or composition of the medium, on the other. A much closer relation seemed to hold between the length of the primary root and the composition of the medium; consequently, in this paper the length of the primary root only is correlated with the chemical and physical data.

Throughout this paper concentrations are expressed as multiples of $M \times 10^{-6}$. M has its usual significance; it indicates a solution containing the gram-molecular weight of a compound in one liter of water. A solution whose concentration is $M \times 10^{-6}$ is therefore of one one-millionth molecular concentration, and a solution ten times as strong, for example, has the concentration $10M \times 10^{-6}$. In our work the least difference in concentration between adjacent cultures of a series containing only univalent ions was $6M \times 10^{-6}$; the greatest was $32M \times 10^{-6}$.

The reader will of course understand that two solutions of the same gram-molecular concentration are not necessarily chemically equivalent. A molecular solution of $Ca(NO_3)_2$, for example, is electrolytically equivalent to a twice molecular KNO_3 solution. This fact should be kept constantly in mind in order to avoid confusion in comparing the curves for the different experiments. It will be noticed that the distilled water curve is much flatter in the case of the bivalent salts than in the case of the univalent salts. This is due to the fact that the concentration of electrolytes excreted by the roots is in the one case expressed in terms of a bivalent salt, in the other case in terms of a univalent salt. In the case of all the univalent salts which we used except KH_2PO_4, the conductance of equimolecular solutions was about the same for all, and roughly half the conductance of the bivalent salts. KH_2PO_4 is exceptional, as may be seen from the curves. It dissociates for the most part as a univalent salt into K^+ and $H_2PO_4^-$ ions, but has a much lower conductance than other univalent salts. The curves are therefore very steep in the figures which deal with KH_2PO_4, and in view of the fact that this salt induced excretion, the shape of the curves can not be regarded as very significant.

There is no reason to doubt that absorption and excretion of electrolytes take place simultaneously in all culture solutions. The figures accompanying this paper indicate of course only the net result of the two processes at any particular time. It would be very desirable to determine the composition of the culture solutions at the close of each experiment, in order to learn the magnitude of excretion into solutions from which there had been a net absorption, and *vice versa*. Thus far, however, we have been unable to make analyses of residual solutions. For the sake of simplicity, we have used the unmodified terms absorption and excretion in the detailed accounts of our experiments, but it should not be assumed that we have ever considered either process to have taken place to the exclusion of the other. Some of the results do indeed indicate that in certain cases the magnitude of excretion is negligible, for at the close of the absorption phase the residual culture solutions have been nearly exhausted of ions.

If a culture solution is of such concentration and composition that absorption by the roots exceeds excretion, the concentration of the solution diminishes from day to day, as long as the vigor of the seedlings remains unimpaired. The length of time that they can live in the dark is, however, conditioned by the reserve materials in the seed. As these materials are depleted the vigor of the seedlings diminishes. After about ten to fourteen days, in the case of *Lupinus albus*, the seedlings weaken, and the absorption phase is succeeded by an excretion phase. The roots rapidly give up their salts to the culture solution and the plants die.

Calcium Nitrate. Experiments 1 and 2

In experiment 1 solutions of $Ca(NO_3)_2$ ranging in concentration from 20 to $260M \times 10^{-6}$ were used. The difference in concentration between neighboring cultures of the series was $20M \times 10^{-6}$. The cultures were under observation 15 days.

Data covering the concentration changes are shown in figure 1. The units on the axis of abscissas indicate duration in days. The ordinates show the concentrations in gram molecules per 10^{-6} liters of distilled water. The course of the curved lines shows concentration changes calculated in gram molecules of $Ca(NO_3)_2$ dissolved in 10^{-6} liters of distilled water. The reader, therefore, can ascertain approximately the concentration of any solution at any time during the experiment and therefore the magnitude of change. The corresponding values of the control experiment in distilled water are represented by the line beginning at zero on the axis of ordinates. It appears that in these solutions of $Ca(NO_3)_2$ the roots of *Lupinus albus* lost electrolytes to the medium during the first day regardless of original concentration, the loss being in general greater in the weaker solutions than in those more concentrated. The increased CO_2 content of the solutions probably accounts in large part for the apparent loss of salts from the roots. The period of apparent loss to the solution was followed in the less concentrated cultures (20 to $60M \times 10^{-6}$) by a day during which no clear change took place. In the medium and stronger concentrations (80 to $260M \times 10^{-6}$) absorption proceeded at a gradually increasing rate. Beginning at about the fifth day in a majority of the cultures absorption proceeded at a relatively uniform rate until about the twelfth or fourteenth day, when the conductivities indicated either a slackening of absorption or a predominant loss of ions from the roots to the solution. The loss of electrolytes was probably due to the changes in the cells of the roots caused by approaching exhaustion from a lack of available food. The root system showed little evidence of deterioration but a withering of the etiolated foliage leaves and even of the edges of the cotyledons took place. The hypocotyl sometimes also broke down.

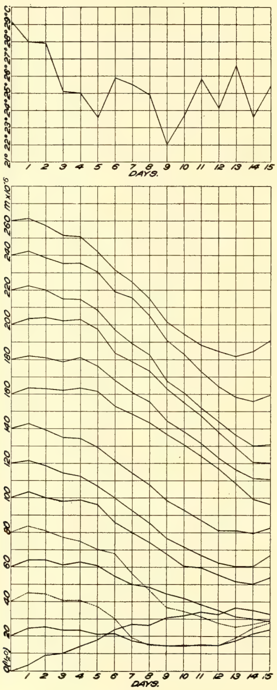


FIG. 1. Curves showing the changes in concentration of the $Ca(NO_3)_2$ solutions used in experiment 1.

The record of the weaker solutions (20 to $80M \times 10^{-6}$) offers an interesting point. In the most dilute solution ($20M \times 10^{-6}$) the roots seemed to be unable to absorb actively at any time leaving the solution little changed in its net ion concentration. In the solutions which had concentrations of 40 and $80M \times 10^{-6}$ respectively the roots were able to absorb actively after a period of delay decreasing in length with the increasing concentration of the solutions. After two weeks of absorption both reached a concentration similar to that seen in the weakest solution, which may perhaps represent the concentration at which $Ca(NO_3)_2$ becomes unavailable to the plants under the given conditions.

The behavior of the distilled water check showed a striking contrast to that seen in the calcium cultures. The roots lost ions with approximate regularity for about 7 days after which the rate of loss decreased for the remaining time. At the close of the experiment the distilled water was equal in its ion content to over $30M \times 10^{-6}$, that is, it was richer in ions than the final concentration of any $Ca(NO_3)_2$ solution which had had an original concentration less than $100M \times 10^{-6}$. It will be seen that in general the net absorption in $Ca(NO_3)_2$ solutions was considerably greater in the stronger solutions than in the more dilute members of the series, due probably to the fact that there was more material present to be absorbed. The maximum absorption in comparison with the amount of $Ca(NO_3)_2$ offered was, as might be expected, highest in the more dilute solutions (20 to $40M \times 10^{-6}$). It seems necessary, if active absorption is to take place, that at least $40M \times 10^{-6}$ $Ca(NO_3)_2$ should be present in the solution, and it is much better if two or three times that concentration is present.

Measurements of various dimensions of the seedlings used were made at the close of the experiment, but only the growth of the primary root seemed to show any perceptible relation to modification of the medium. The growth made in distilled water was distinctly poorer than that seen in any of the calcium solutions and in a general way the total growth in length made by the roots was greater as the salt content of the medium increased. In some cases this was not true, but in view of the small number of plants in each culture there is good reason to believe that these departures were due in considerable part to the individual variation of the plants.

In view of the results seen in the experiment summarized in figure 1, it was thought desirable to ascertain the effect of further dilution of the salt and at the same time repeat the observations as far as the lesser concentrations were concerned. Accordingly in experiment 2 a series of thirteen solutions was made up ranging from 8 to $104M \times 10^{-6}$ with a constant interval of $8M \times 10^{-6}$ between adjacent members of the series. The experiment ran seventeen days, and is summarized in figure 2.

The record of these solutions shows the same general features seen in the foregoing experiment. The lupine roots were always able to make some absorption, leaving even the weakest solution poorer in ions than it was at the beginning of the experiment. In general, net absorption increased with the increase of the original salt content up to a concentration of $40M \times 10^{-6}$. In this and in the more concentrated solutions the maximum absorption varied somewhat but did not advance with the increasing salt content. This seems to mean that the demand for this salt under the conditions to which the plants were subjected was satisfied by a concentration of $48M \times 10^{-6}$.

The growth rate in general increases with the concentration in the more dilute members of the series and reaches a general level which with considerable variation is maintained. This general level is attained at the point of maximum absorption.

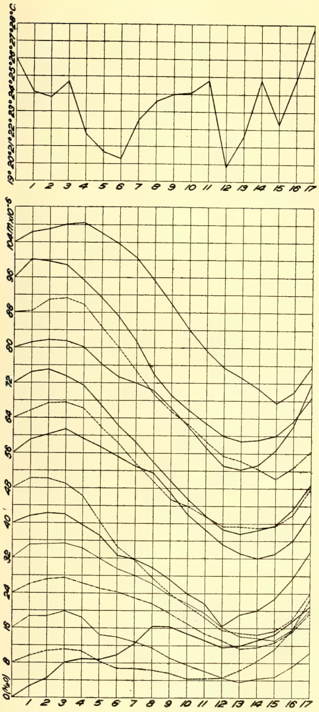
CALCIUM SULPHATE. EXPERIMENT 3

Fourteen solutions of calcium sulphate were studied, in concentration ranging from 12 to $168M \times 10^{-6}$, the regular interval between adjacent members of the series being $12M \times 10^{-6}$. The experiment ran seventeen days, during which time great temperature variations were noted.

The curves presented in figure 3 show a very active absorption by the roots in all the solutions, excepting only the distilled water check. As usual, the distilled water extracted electrolytes from the roots until a fairly constant concentration was reached at which excretion was perhaps balanced by the reabsorption of the same ions. The phase of balance between the two processes was followed by the usual increasing leakage of electrolytes from the roots as they approached exhaustion.

In all $CaSO_4$ solutions from 12 to $72M \times 10^{-6}$ absorption was active, beginning in the upper part of this range on the first day of the experiment. Under the influence of this absorption the ion concentration of the six weaker solutions fell rapidly to a point approaching on about the tenth day that of the distilled water at that time and becoming much weaker in ions toward the close of the experiment than even the distilled water itself. It is probable that in these more dilute solutions the roots absorbed practically all the salts they could and reduced the concentration to the unavailable minimum. This unavailable minimum was represented by a concentration of $CaSO_4$ from 12 to $18M \times 10^{-6}$. A culture containing 500 c.c. of a $72M$

$\times 10^{-6}$ solution of $CaSO_4$ was reduced in concentration through absorption to a little over $12M \times 10^{-6}$, and provided four lupine



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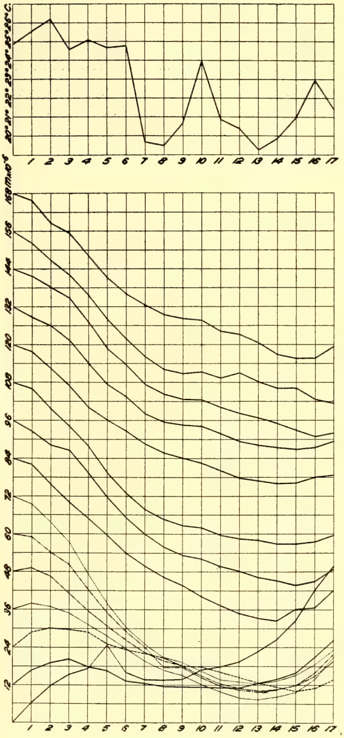

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FIG. 2. Curves showing the changes in concentration of the $Ca(NO_3)_2$ solutions used in experiment 2.

FIG. 3. Curves showing the changes in concentration of the $CaSO_4$ solutions used in experiment 3.

seedlings with all the $CaSO_4$ which they were able to absorb during their short life in the dark. In the cultures which offered an increased salt concentration the maximum net absorption did not rise, seeming to indicate a surplus of calcium sulphate over the quantity which could be absorbed.

The root growth in the more dilute solutions seemed to increase with the increase in $CaSO_4$ concentration but in the more concentrated solutions no increasing root growth was apparent. In general there was a rough parallelism between root growth and absorption of $CaSO_4$. Neither increased indefinitely as the initial concentration of the culture solution was increased.

Magnesium Nitrate. Experiments 4 and 5

In experiment 4 thirteen solutions of $Mg(NO_3)_2$ were used ranging in concentration between 8 and $104M \times 10^{-6}$ with a constant difference of $8M \times 10^{-6}$ between members of the series. In view of the markedly toxic properties of magnesium salts the concentrations used were less than in the case of the calcium salts. The course of the experiment covered fourteen days. Here, as in the $Ca(NO_3)_2$ solutions, the roots were unable to absorb electrolytes during the first day in a quantity sufficient to make up for the real and apparent losses. In the weaker solutions (8 to $24M \times 10^{-6}$) the loss preponderated over such possible absorption as may have taken place so greatly for the first four days as to leave the solutions markedly richer in ions than at the beginning of the experiment. It will be noted that the net loss of ions from roots was greater in the most dilute solutions (8 and $16M \times 10^{-6}$). In the more concentrated members of the series, absorption predominated more promptly over loss and at a generally increasing ratio as the concentration increased. This was seen both in the shortening of the preliminary period of apparent loss and in the greater rapidity of absorption during comparable intervals. In general, absorption began to assume its most rapid rate after the elapse of about four days and continued to show this maximum rate for the succeeding seven or eight days. At about the twelfth day, or, in the more dilute members of the series a day earlier, the roots began to lose ions more rapidly than they absorbed them, a reversal of action due probably to approaching exhaustion. This loss rapidly increased and the more dilute solutions at the close of the experimental period were richer in electrolytes than at the beginning.

An instructive accident happened in the culture containing a concentration of $80M \times 10^{-6}$. After showing the usual record of absorption for eight days, the solution on the ninth day showed a marked increase in ions. This increase was continued at a rapid and rather uniform rate till the close of the experiment. An examination at that time showed that one plant was injured at a point near the surface of the solution and by the leaching of electrolytes from the dead tissues had furnished a source of contamination.

A study of the growth of the primary roots in the different solutions of $Mg(NO_3)_2$ showed that while in the lower and higher concentrations the rate was about equal to that seen in distilled water, a better growth prevailed in the medium concentrations (32 to $72M \times 10^{-6}$) with a fairly well marked maximum in solutions 48 to $64M \times 10^{-6}$. This region of better growth coincided fairly well with that in which the maximum absorption took place. There were many evidences of toxic action in the stronger concentrations. The great reduction of the laterals, the development on the primary roots of occasional discolored tissue and the frequent appearance of reddish blotches at the bases of the hypocotyls may be mentioned here. These characteristics of Mg action will be again referred to in connection with another experiment involving higher concentrations in which these features come out more clearly.

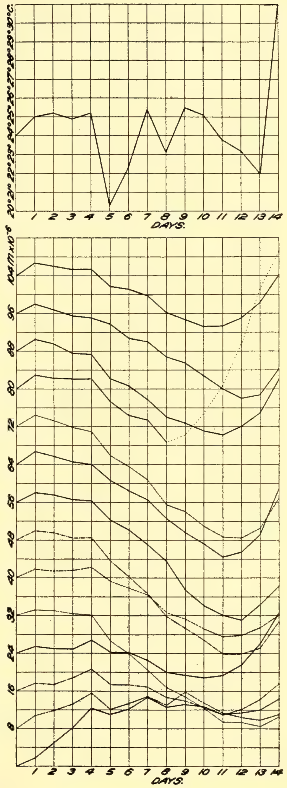


FIG. 4. Curves showing the changes in concentration of $Mg(NO_3)_2$ solutions used in experiment 4.

In experiment 5, a second series of thirteen solutions of $Mg(NO_3)_2$ was used in concentrations lying between 12 and $156M \times 10^{-6}$, with a constant interval of $12M \times 10^{-6}$ between members of the series. The experiment lasted 16 days. The results are plotted in figure 5.

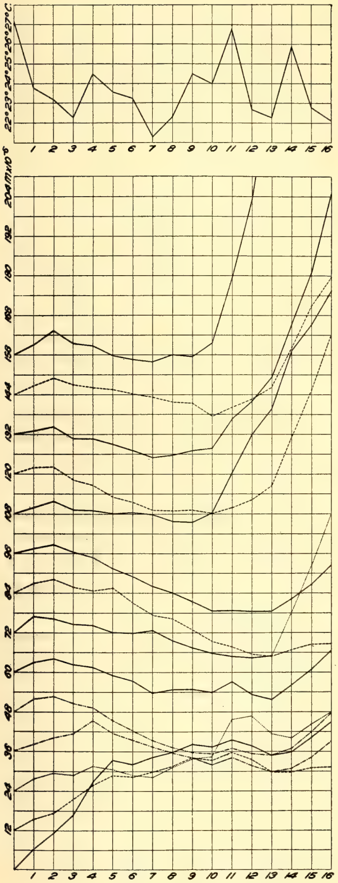
The same general characteristics noted in experiment 4 are again seen in this experiment and need not receive special mention. It will be noted in those concentrations exceeding the maximum salt content of experiment 4 that the toxic action of the salt became more marked, as was seen in the earlier onset of the antemortem leaching phase. The practical disappearance of an absorption phase in solutions about $96M \times 10^{-6}$ probably indicates the same thing.

An interesting point to be noted in both experiments is the reduction of the weaker solutions to a common salt content. In experiment 4 on the tenth and eleventh days the weakest four $Mg(NO_3)_2$ solutions and the distilled water check agreed in having an ion content of about $12M \times 10^{-6}$ calculated as $Mg(NO_3)_2$. Since the $32M \times 10^{-6}$ solution was reduced to $12M \times 10^{-6}$ the net reduction in concentration due to salt absorbed was approximately $20M \times 10^{-6}$. In some of the higher concentrations the reduction was somewhat greater. In experiment 5 a similar situation was seen. On the ninth and tenth days the weakest four salt solutions and distilled water had a like ion concentration, representing in the strongest of these four salt solutions a reduction of about $12M \times 10^{-6}$. This is somewhat greater than the average reduction in the solutions containing a considerable surplus of the salt. Again the best growth and the greatest absorption were found in a general way to coincide.

MAGNESIUM SULPHATE. EXPERIMENT 6

Magnesium sulphate solutions were made up in fourteen different concentrations, running from 12 to $168M \times 10^{-6}$, the regular interval being $12M \times 10^{-6}$. The experiment ran thirteen days.

The results graphically presented in figure 6 show that the action of magnesium sulphate is essentially the same as that of the nitrate. Those solutions having the least salt caused the excretion of electrolytes from the roots. As the concentration increased to somewhat more than $24M \times 10^{-6}$ the roots were able to absorb, this absorption rising somewhat with the increase in concentration until a salt content equal to about $120M \times 10^{-6}$ was reached. Beyond this point, a further increase of salt was not accompanied by any increase of absorption. There was a tendency in the higher concentrations here used toward a reduction of absorption, due probably to the toxic



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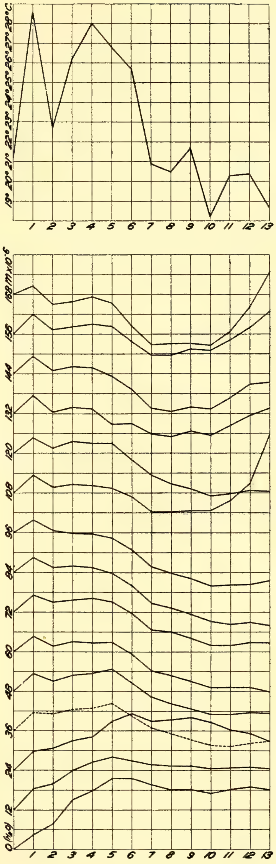

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FIG. 5. Curves showing the changes in concentration of the $Mg(NO_3)_2$ solutions used in experiment 5.

FIG. 6. Curves showing the changes in concentration of the $MgSO_4$ solutions used in experiment 6.

action of the salt. This supposition was supported by the decreased root growth and by the appearance of pathological symptoms characteristic of Mg injury. In a mild degree of injury the xylem at the base of the hypocotyl and more especially in the upper part of the primary root showed a more or less extensively developed yellowish-brown discoloration. As the injury became more serious this staining of the tissues worked to the surface near the union of the radicle and the hypocotyl and appeared as a blotch of reddish-brown color. In the stronger solutions the surface of the radicles showed sunken areas having a reddish-brown color. In this experiment the staining of the xylem appeared in the roots even in the most dilute magnesium sulphate solution. The blotching at the union of the hypocotyl and primary root came clearly into view in the $48M \times 10^{-6}$ solution and in the concentrations showing the maximum absorption, the injury was very marked and the development of lateral roots was almost suppressed.

The course of the curve showing the average growth made by the roots indicated little tendency on the part of the $MgSO_4$ to help growth, the result showing at no point any marked superiority over the growth in the distilled water check.

$MgSO_4$ seemed to act like calcium in so far that it permitted the roots to make a net absorption, but at the concentrations most favorable to absorption the toxicity interfered with the health of the root. Thus the usefulness of Mg, in the absence of other + ions, seems to depend on its occurrence in great dilution.

The fact that $Mg(NO_3)_2$ and $MgSO_4$ closely parallel each other in their action seems to make it clear that the specific characteristics seen in both are preponderantly due to the Mg ion.

Potassium Nitrate. Experiment 7

The series of thirteen solutions of potassium nitrate studied increased in concentration from 32 to $416M \times 10^{-6}$ with an interval of $32M \times 10^{-6}$ between members of the series. The experiment ran fifteen days.

The results presented graphically in figure 7 show that potassium nitrate exerted an action on lupine roots markedly different from that seen in the cases of calcium and magnesium salts. In the weaker solutions (32 to $160M \times 10^{-6}$) the plants behaved very much as they do in distilled water. During the first four or five days the roots showed a marked loss of electrolytes, this loss decreasing somewhat in magnitude as the concentration of the solution increased. After this preliminary period of steady loss, the roots seemed to gain the ability to resist the action of the solutions and during the remaining ten days maintained themselves without affecting in a decided way the electrolytic concentration of the media. The loss of ions which took place during the first four or five days remained as the net result of the ionic exchange between the solutions and the roots.

In the solutions of higher concentration, a similar period of preliminary loss was seen, diminished, however, in comparison with that seen in the more dilute members of the series. The following recovery was, however, more complete and the roots were often able to absorb sufficiently to bring the solution back to approximately the original concentration. In some cases the absorption phase developed sufficiently to show a net absorption by the roots prior to the setting in of the final leakage. Such a result was seen in the cultures growing in solutions of the original concentrations 224, 228, 320, and $352M \times 10^{-6}$. Such a net gain, however, never quantitatively equalled that seen in $Ca(NO_3)_2$. The roots of Lupinus with difficulty maintained a net absorption in KNO_3 solutions.

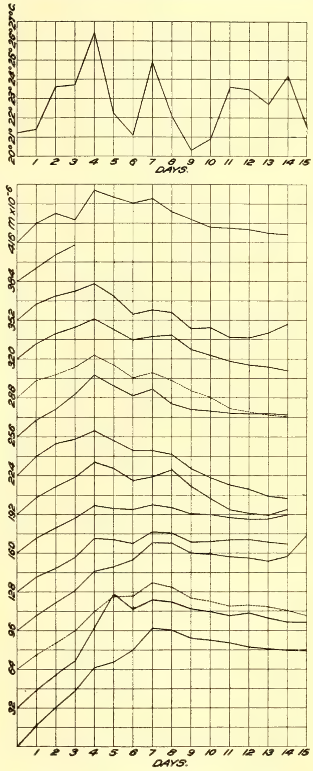


FIG. 7. Curves showing the changes in concentration of the KNO_3 solutions used in experiment 7.

This result was reflected, to a great degree, in the growth of the roots placed in these solutions. Poor growth was the rule in the solutions which show a strong extraction of ions from the plant, a slight improvement being seen in those solutions in which a more active absorption took place. The solutions of KNO_3 seemed furthermore to induce or accompany a general debility in the seedlings. This was shown by the large number of plants that for reasons not obvious deteriorated in the cultures. Individuals developing increased leakage due, perhaps, in part to injury not marked enough to lead to detection and removal were probably largely responsible for the irregularity of the results. The $256M \times 10^{-6}$ solution compared with the neighboring solutions showed an aberrantly high loss of ions from the roots and probably represented the partial failure of one or more individuals in the culture.

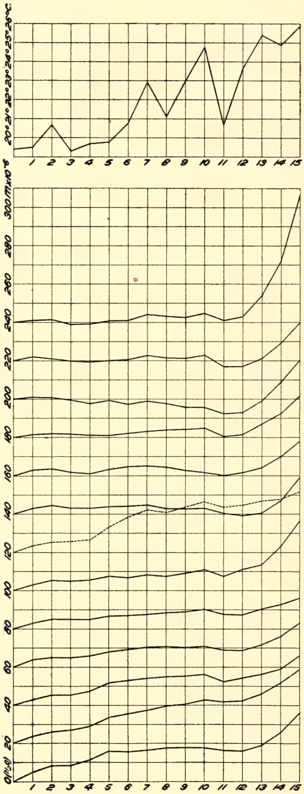


FIG. 8. Curves showing the changes in concentration of the K_2SO_4 solutions used in experiment 8.

POTASSIUM SULPHATE. EXPERIMENT 8

Twelve solutions of K_2SO_4 were made varying in concentration from 20 to $240M \times 10^{-6}$, the concentration interval being $20M \times 10^{-6}$. The experiment ran fifteen days. The results are plotted in figure 8.

Potassium sulphate shows a physiological action very similar to that of potassium nitrate. In the more dilute solutions, 20 to $120M \times 10^{-6}$, the plants lost ions to the medium in a measure approximately like that seen in the corresponding nitrate series. In both salts an absorption phase appeared in the more concentrated solutions.

In K_2SO_4 a small net absorption was seen in concentrations ranging from 140 to $204M \times 10^{-6}$. The similarity of the records of the more dilute solutions of K_2SO_4 to that of the distilled water check is seen at a glance. In the higher concentrations an equilibrium seemed to be immediately established between the roots and the solution, the result being seen in the slight change in ion concentration which took place until the leakage due to approaching collapse set in.

The growth made by the roots of seedlings grown in these solutions was equal to or less than that seen in distilled water.

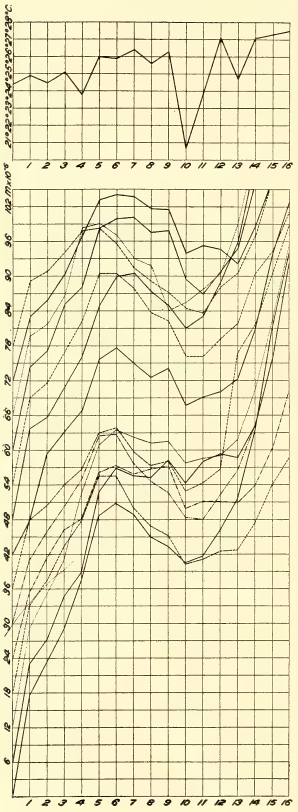
POTASSIUM DIHYDROGEN PHOSPHATE. EXPERIMENTS 9 AND 10

The range of concentration of solutions of potassium phosphate studied lay between 6 and $208M \times 10^{-6}$, the fixed interval between members of the series being $6M \times 10^{-6}$ in experiment 9 and $16M \times 10^{-6}$ in experiment 10. The experiment was continued fifteen days in the stronger solutions, sixteen or seventeen days in the weaker ones. The results are graphically shown in figures 9 and 10.

The striking fact shown by these curves is an almost unrelieved loss of salts by the seedlings in all concentrations tested. During the first four or five days a period of rapid gain in concentration by the medium was seen, followed by a period of slackened loss by the plants. Even in this second period, which corresponds in the salts previously studied to the time of most rapid absorption, the usual result seen is an interval in which a more or less well marked equilibrium was maintained. In 64, 96, 176, and $192M \times 10^{-6}$ solutions, absorption predominated by a narrow margin for several days, but in the other members of the series the loss of ions by the roots was the rule. The final period of loss by the plants due to approaching breakdown was a very marked feature, at times an exaggerated one.

In every solution of the series the result was a marked net loss of ions by the plants. In none was this net loss so great as in the distilled water check, but in the weaker solutions the leaching of the roots seemed to be almost as severe.

The growth made by the roots in no case showed any considerable advance over that made in distilled water. Indeed, the whole picture presented by this salt in the concentrations here studied strikingly resembled that presented by distilled water. Further evidence, showing the harmful action of KH_2PO_4, was seen in the loss of about 15 per cent. of the plants used in the cultures. In several cases entire cultures had to be abandoned.



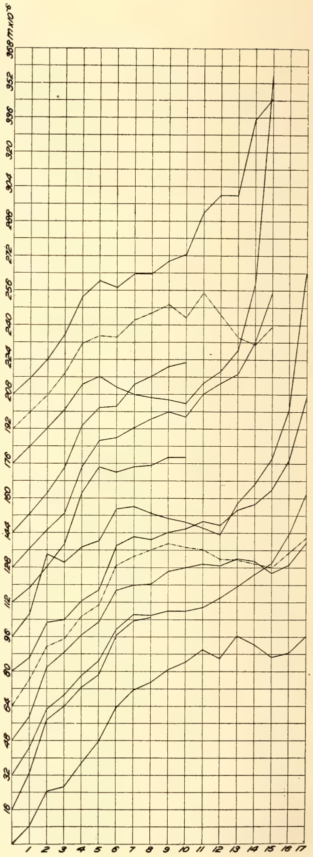


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FIG. 9. Curves showing the changes in concentration of the KH_2PO_4 solutions used in experiment 9.

FIG. 10. Curves showing the changes in concentration of the KH_2PO_4 solutions used in experiment 10.

Potassium Chloride. Experiment 11

The series of solutions of this salt, thirteen in number, covered concentrations ranging from 32 to $416M \times 10^{-6}$ with a regular difference of $32M \times 10^{-6}$ between concentrations. The experiment ran fourteen days. The results are shown in figure 11.

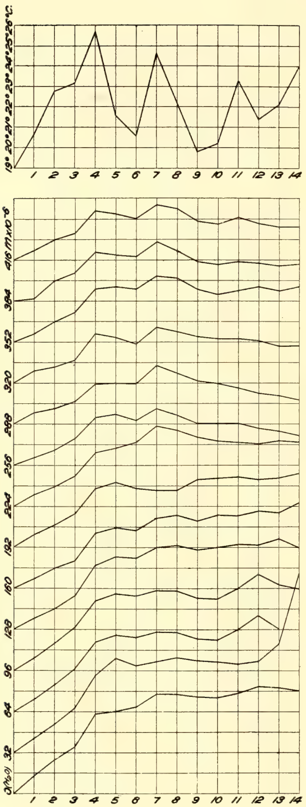
The record of these solutions showed a quite remarkable similarity of behavior regardless of the concentration as is shown by the parallel course of the curves. The result is the more striking when it is noted that the solution curves not only parallel each other but very greatly resemble that of the distilled water control. No clear absorption took place in any solution until a concentration of $224M \times 10^{-6}$ was reached. In this concentration and the stronger ones, up to $320M \times 10^{-6}$, there was slight absorption during the latter part of the period. In general the roots in KCl solutions may be said to have lost heavily for four days after which the net loss of ions still proceeded at a slower rate, at times with the maintenance of an equilibrium for several days. In a few cases slight gains of absorption over leakage were seen but generally the loss preponderated over the gain. In no culture did the total absorption approach in magnitude the total loss. The action of KCl solutions is strikingly like that of distilled water, differing, however, in that the higher members of the series bring about a less marked initial loss of ions from the roots.

The growth of the primary roots showed a marked gain over that in distilled water in but one culture, $128M \times 10^{-6}$. In its effect on the growth of roots, KCl in the concentrations studied acted essentially like distilled water.

Sodium Chloride. Experiments 12 and 13

A comparison of the action of Na with that of K under the conditions of these experiments being desired, two experiments were carried out with NaCl. In experiment 12 thirteen solutions of sodium chloride were prepared ranging in concentration from 8 to $104M \times 10^{-6}$ with a regular interval of $8M \times 10^{-6}$ between members of the series. The experiment ran fifteen days. The curves are shown in figure 12.

An inspection of the record of these cultures shows that NaCl is very similar in its action to several potassium salts. After an initial period of three days during which the roots in all solutions gave up ions an absorption phase set in which reached its maximum on the



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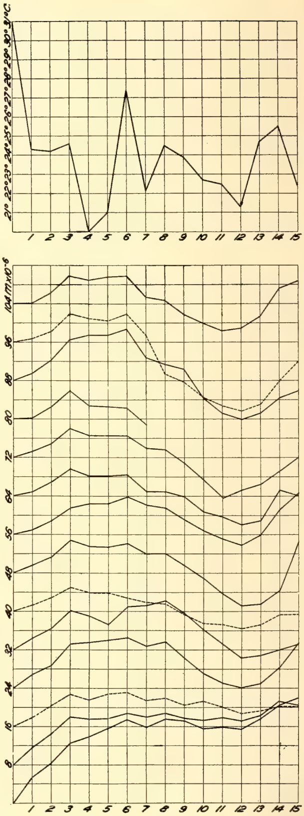

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FIG. 11. Curves showing the changes in concentration of the KCl solutions used in experiment 11.

FIG. 12. Curves showing the changes in concentration of the NaCl solutions used in experiment 12.

eighth to twelfth day. This absorption phase in all except the more dilute solutions resulted in a very slight net absorption before the final escape of salts due to exhaustion set in. In the more dilute members of the series, 8 to $16M \times 10^{-6}$, the solutions through absorption came to a level then prevailing in the distilled water control culture, viz., about $20M \times 10^{-6}$. This record recalls most forcibly the situation found in solutions of KNO_3 and K_2SO_4.

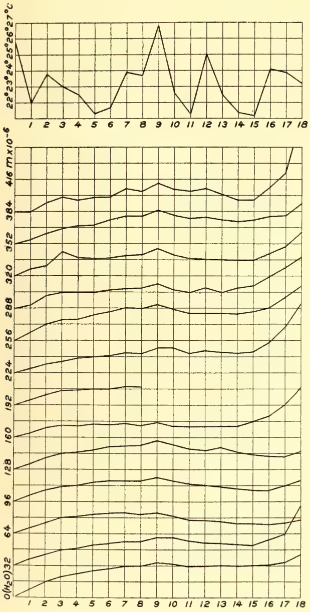

FIG. 13. Curves showing the changes in concentration of the NaCl solutions used in experiment 13.

A somewhat similar experiment dealing with this same salt was carried out in a series showing a somewhat wider range of concentrations. Experiment 13 covered 12 dilutions of NaCl ranging from 32 to $384M \times 10^{-6}$, with a regular interval of $32M \times 10^{-6}$ between members of the series. The experiment ran eighteen days. The results are plotted in figure 13.

In this series during an initial period of about three days the solutions increased more rapidly in ions than was the rule during the following ten to twelve days. This second period was marked in the weaker solutions by a poorly developed absorption phase from about the thirteenth to the sixteenth day. The withdrawal of ions, however, was never sufficient to overcome the previous accessions and in no solution was a net absorption seen, although in no case was the net deficit very great. On the fourteenth to sixteenth day, varying with the individual culture, marked gain in the ion content was seen in nearly all solutions, the losses marking the exhaustion of the vigor of the plant.

The general behavior of this series again suggests that of certain of the potassium salts. The results of these experiments with NaCl seem to group this substance with those in which root absorption is greatly reduced and to suggest a close parallel between the NaCl solutions and distilled water.

The growth of the roots in the NaCl solutions here tested was depressed to or below the point characteristic of distilled water.

Conclusions

1. Roots of *Lupinus albus* grown in darkness in distilled water give up their salts to the water at a varying rate until the death of the plants occur through exhaustion of the reserves.

2. KH_2PO_4 and KCl solutions act essentially like distilled water in the concentrations studied by us. Solutions of K_2SO_4 and KNO_3 show a slight absorption phase resulting in a minimal net gain in salts to the plant but otherwise differ little from the phosphate and chloride.

3. Absorption and growth take place in NaCl essentially as in KNO_3 and K_2SO_4.

4. Solutions of $Mg(NO_3)_2$ and $MgSO_4$ support a slight but clearly developed absorption phase resulting in a net gain in salts to the plant. A net leakage of salts is seen in the more dilute solutions, and toxic action in those of greater concentration.

5. Calcium nitrate and calcium sulphate in all concentrations studied are actively absorbed by the roots and apparently enable the plants to retain possession of the salts already present.

Bureau of Plant Industry,
Washington, D. C.

NOTES ON THE FORMS OF CASTELA GALAPAGEIA

ALBAN STEWART

According to the Index Kewensis, the genus Castela Turp. is composed of eleven species, which occur for the most part in Mexico, West Indies, and southern South America, the genus being thus distinctly American in its distribution. One of these species, *C. galapageia* Hook. f., is peculiar to the Galapagos Islands and was described from material collected by Darwin when he visited the Islands on his voyage around the world as naturalist of the Beagle. Darwin collected his material on Chatham Island, and it was not known until nearly sixty years afterwards, when Baur visited the Galapagos Islands, that this species occurred on other islands of this group. Baur succeeded in finding this species on Albemarle, Bindloe, Charles, Chatham, Duncan, Jervis, and James Islands, and the collections made by him were studied by Robinson and Greenman[1] of the Gray Herbarium of Harvard University. Eight years after Baur was on the islands, they were again visited by Snodgrass and Heller of the Hopkins-Stanford Expedition. Their collections of plants were the most complete that had been taken from the islands up to that time, but they succeeded in extending the range of *C. galapageia* to but one other island, viz., James.

The collections of Snodgrass and Heller were studied by Robinson, who, aided by the additional specimens which this collection furnished, thought that he could recognize formal differences in the Castela material. He consequently described six new forms[2] basing his differences mainly on the arming of the stem and the shapes of the leaves. The forms described by Robinson are: albemarlensis, bindloensis, carolensis, duncanensis, jacobensis, and jervensis, the forms being named for the various islands respectively on which the type specimen of each was collected.

I was fortunate enough to secure much additional material of this species when I visited the Galapagos Islands in 1905–1906 as botanist of the expedition sent hence by the California Academy of Sciences, as well as to extend its range to Abingdon, Barrington, Hood, Indefatigable, Narborough, and Seymour, so that this species is now known to occur on all of the larger islands of the group. After returning from the expedition I studied the collections and prepared the results for publication at the Gray Herbarium. I felt some doubt about the distinctness of the forms described by Robinson after I had compared the Academy specimens with his material. In fact I was convinced that the form jervensis did not differ from the form albemarlensis, and I considered it as such in my work upon the flora of these islands.[3] The time at my disposal then would not allow me to make a critical study of this species so that it had to be abandoned until now.

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[1] On the Flora of the Galapagos Islands as Shown by the Collections of Dr. Baur. Amer. Journ. Sci. III., **50**: 135–149. 1895.

[2] Flora of the Galapagos Islands. Proc. Amer. Acad. **38**: 77–269, pls. 1–3. 1902.

FORMA ALBEMARLENSIS Robinson

This form is described as being unarmed or armed with small slender spines. Leaves oblong obtuse mucronate, 1.5–3.4 cm. long, very often furnished with 1–2 small lateral teeth; not shiny above. After comparing the Academy material with the type specimen of this form, I succeeded in a way in matching specimens from Albemarle, Indefatigable, and Narborough Islands with it. The specimens from these islands can be separated into two groups, viz., those which have the stem unarmed, and those which have the stem armed with spines to a greater or less extent. In the unarmed group are specimens from Tagus Cove and Villamil, Albemarle Island, and from the northeast and southeast sides of Indefatigable Island, while in the armed group are specimens from the northwest side of Indefatigable and from Narborough Islands. The armed specimen from Indefatigable Island is provided with short spines which are somewhat thickened at the base, while the specimen from Narborough Island has but one spine on it which is slender and nearly 7 mm. long.

Great variation occurs in the leaf characters of the different specimens, well illustrated in figure 1, which consists of tracings of leaves taken from a specimen collected at Tagus Cove, Albemarle Island. Leaves which are distinctly of the oblong obtuse mucronate type, similar to those described as being typical of this form, occur on this specimen, figure 1 *a*. In addition to this kind of leaf, however, there are still others, which are rather oblanceolate mucronate, figure 1 *b*;

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[3] A Botanical Survey of the Galapagos Islands. Proc. Calif. Acad. IV., 1: 82. 1911.

lanceolate mucronulate, figure 1 *c*; spatulate slightly retruse, figure 1 *d*; and obovate retruse mucronate, figure 1 *e*. The leaves are slightly shiny on the upper surface, but as this is a character which seems to vary with the age of the specimen and the care with which it was dried, it can hardly be considered as distinctive. It might be added that, after comparing this specimen with the type specimen of this form, I find that there are leaves very similar in shape to those shown in figure 1*a*, *c*, and *d*, to be found upon it. The margins of many of the leaves on the Academy specimen are somewhat revolute.

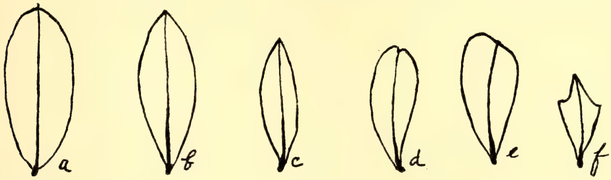

Fig. 1

The specimen from Villamil Albemarle Island has many leaves which are oblong obtuse mucronate, figure 2*a*.

There are still other leaves which are obovate and distinctly retruse, figure 2*b*, and still others which are spatulate, similar to those shown in figure 1*d*. In fact the bases of the most of the leaves on this specimen are rather too attenuate at the base to be considered oblong, approaching more a spatulate shape in this respect. The margins of the leaves are slightly revolute in many instances, as was the case with the specimen from Tagus Cove.

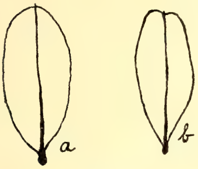

Fig. 2

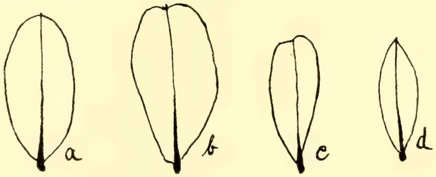

Fig. 3

The specimen from the southeast side of Indefatigable Island has more of its leaves oblong obtuse mucronate in shape than any of the other unarmed specimens assigned to this form. A typical leaf of this kind is shown in figure 3*a*.

In addition to this kind of leaves, there are others which are obovate slightly retruse mucronate, figure 3*b*; spatulate retruse, figure 3*c*; and broadly lanceolate, figure 3*d*. The margins of many of the leaves are slightly revolute.

The specimen from the northeast side of Indefatigable Island has no leaves which are distinctly oblong, as the bases are rather attenuate in nearly every instance. There are a few leaves, however, which approach in shape those described by Robinson, one of which is shown in figure 4*a*. Other kinds of leaves on this specimen are oblanceolate, figure 4*b*; spatulate mucronate, figure 4*c*; and cuneate, figure 4*d*.

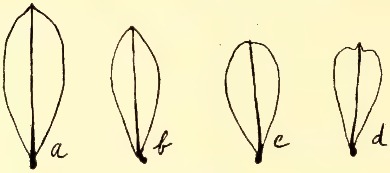

Fig. 4

Many of the leaves of this specimen have strongly revolute margins, a condition that may be brought about by the loose nature of the soil and the extreme dryness of this part of the island. The upper surfaces of many of the leaves are wrinkled, a character which is present to a greater or less extent in many of the Academy specimens. It seems to vary with the age of the leaves as does the character of shininess.

The armed specimen from the northwest side of Indefatigable Island[4] has many leaves which are typical as is shown in figure 5*a*. Other kinds of leaves on this specimen are: oblong retruse, figure 5*b*;

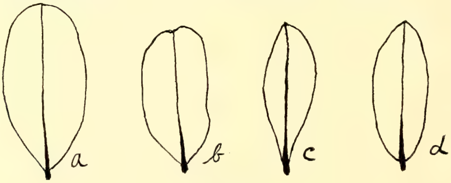

Fig. 5

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[4] Owing to a typographical error this specimen No. 1749 was listed as being from the northeast side of Indefatigable Island in my former work on the Galapagos flora. L. c., p. 82.

oblanceolate acute mucronate, figure 5*c*; and elliptical mucronate, figure 5*d*. The margins of many of the leaves are revolute.

The leaves on the armed specimen from Narborough Island, taken as a whole, are rather oblong in shape, but with few exceptions they are acute at the apices. The leaves shown in figure 6 are rather typical of this specimen, *a* being oblong acute mucronate, *b* oblong elliptical, similar in size and shape, by the way, to a leaf of the type specimen of this form, and *c* is spatulate.

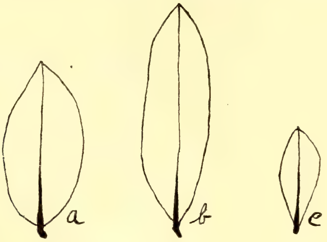

Fig. 6

Strange to say, none of the Academy specimens have the small lateral teeth on the leaves which Robinson[5] mentions as being sometimes present. They are quite evident on the specimen collected by Snodgrass and Heller at Tagus Cove, figure 1*f*, but do not occur on the specimen collected by Baur at the southern end of Albemarle Island, evidently in the vicinity of Villamil.

FORMA BINDLOENSIS Robinson

This form is described as being unarmed, leaves entire obovate rounded or mucronulate, 1–1.6 cm. long; not shiny above. The Academy specimen from Bindloe Island does not agree with this description in several ways, as the stem is armed with rather thick short spines, and the leaves vary quite as much in shape as on the specimens assigned to the form albemarlensis which has just been considered. Leaves similar in shape to the one shown in figure 7*a* occur on the specimen, which are evidently like those described by Robinson except that the apices are slightly retruse. There are leaves which have other shapes, however, such as elliptical, figure 7*b*; spatulate both acute and obtuse, figure 7*c* and *d*; and oblong obtuse mucronate, figure 7*e*, which, by the way, is the characteristic shape of the leaves on the form albemarlensis. I find that leaves of this shape sometimes occur on the type specimen of bindloensis, a tracing from one of which is shown in figure 7*f*. There are also leaves which are spatulate and rather oblanceolate on the type specimen of this form. There is a tendency for the margins of the leaves to be revolute in both the type, and the Academy specimen.

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[5] L. c. p. 158.

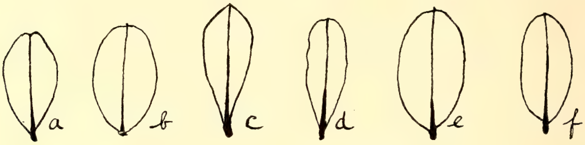

Fig. 7

Forma carolensis Robinson

Described as being armed with short robust spines. Leaves spatulate with manifestly attenuated bases. Upper surface very shiny, 2–2.5 cm. long. Specimens from Abingdon, Charles, Chatham, and South Seymour Islands were assigned to this form in my former work on the Galapagos Island flora.[6] From what has been stated above, it can be seen at once, that, except for the arming of the stem, this description might apply equally well for the type specimen os bindloensis, as spatulate leaves are to be found on it.

There is a great difference in the arming of the stem in the Academy series of specimens, not only from different islands but from the same island as well. Two specimens, assigned to this form in my earlier paper, were collected at Post Office Bay on Charles Island. One of these specimens has spines 1–2.5 cm. long, and it is only on the younger branches that the spines are short. The second specimen from this locality has slender spines, which are somewhat shorter than on the above specimen just described. On neither one of these are the spines short and robust. The type specimen appears to be an old branch so that the shortness of the spines on it can not be due to immaturity.

The spines on the specimen from Abingdon Island are short and robust in some instances while in others they are nearly 1 cm. long.

[6] L. c. p. 83.

The specimen from Chatham Island has short and robust spines, while the specimen from South Seymour is unarmed, as is the type specimen of the species collected on Chatham Island by Darwin.

The leaf characters on this series of specimens are quite variable. The specimen from Abingdon Island has many spatulate leaves, and still others that are rather oblong elliptical, or lanceolate, figure 8.

The most of the leaves are rather acute at both base and apex approaching in this respect the form duncanensis as described by Robinson. There is quite as much variation in the leaf characters of the specimens from Charles Island as there is in the manner of arming of the stem. The leaves are broadly spatulate for the most part on one of these specimens, while on the other they are much narrower, being lanceolate or even linear in some instances. They probably approach closely in shape the leaves on the type specimen of the species as described by Hook. f.

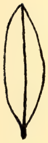

Fig. 8

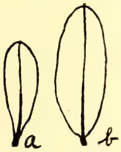

Fig. 9

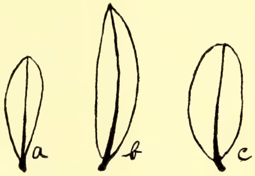

Fig. 10

There are many spatulate leaves on the specimen from Chatham Island, figure 9*a*, but there are others which are oblong obtuse mucronate, figure 9*b*, so that this specimen might be assigned equally well to either of the forms albemarlensis or carolensis on leaf characters, as it has the types of leaves of each. The specimen from South Seymour Island has spatulate leaves, figure 10*a*, those which are lanceolate, figure 10*b*, and also those which are oblong obtuse. Spatulate leaves, described as being typical of this form, also occur on the type specimenf of both the forms albemarlensis and bindloensis as has been mentioned above. It might be well to state in this connection that leaves of this shape occur to a greater or less extent on the Academy specimens from all of the islands where this species was collected.

FORMA DUNCANENSIS Robinson

Described as being unarmed. Leaves entire narrow lanceolate, acute at base and apex. Specimens from Barrington, Duncan, and Jervis Islands have been assigned to it.[7]

Except for the spine-like endings of the lateral branches, the specimen from Barrington Island is weakly armed, but hardly so much as is the specimen from Jervis Island where the spines are very weak indeed. The specimen from Duncan Island has very heavy spines. Notwithstanding the fact that this form is described as being unarmed, there is nevertheless a single weak spine on the type specimen, which is apparently a young and immature branch. The spine might easily be overlooked as it is somewhat hidden by leaves, the specimen being fastened to a herbarium sheet.

The leaves on the Academy specimen of this form from Barrington Island are very small and for the most part are oblanceolate or spatulate in shape with strongly revolute margins. The bases of the leaves may be either obtuse or acute, and the apices are sometimes obtuse. The specimen from Duncan Island in the Academy collection has leaves which for the most part are narrowly spatulate or oblanceolate in shape. In some instances they are nearly linear, a condition apparently brought about by the strongly revolute margins of the leaves. The bases and apices may be either acute or obtuse, both kinds of leaves occurring on the same specimen. The specimen described by Robinson as typical is immature and the leaves are all lanceolate. There is upon the same herbarium sheet, with the type specimen of the form duncanensis, still another specimen, No. 42 (hb. Gr.) from Charles Island which Robinson[8] assigns to the form carolensis with a question. I can find but little difference in these two specimens as the leaves are practically identical in shape on the two. Lanceolate leaves occur on both the type and the Academy specimens of the form albemarlensis as noted above. The leaves on the specimen from Jervis Island are spatulate for the most part, and the leaf margins are less inclined to be revolute than on any other of the Academy specimens assigned to this form. Leaves which are spatulate in shape are thus seen to occur on all of the Academy specimens assigned to this form, and they also occur on all three of the forms previously considered, viz., albemarlensis, bindloensis, and carolensis.

[7] Stewart, op. cit. 83.

[8] Op. cit. 159.

FORMA JACOBENSIS Robinson

Described as being armed with very short spines. Leaves broadly oblong entire, rounded at base and apex, mucronate, shiny above and transversely wrinkled, 1–1.8 cm. long. It can readily be seen that this description is very similar to that given for the form albemarlensis. The only essential difference between these two descriptions is that the leaves in albemarlensis are somewhat larger and are often furnished with one to two lateral teeth as shown in figure 1*f*. The presence of lateral teeth on the leaves is a rare character, however, as shown by the series of Academy specimens, and the size of the leaves varies greatly even on the same specimen. Spatulate leaves, and those which are lance-oblong in shape occur on the type mentioned. The shininess and wrinkled character of the leaves are not distinctive as mentioned above.

From the above discussion it can be readily seen that characters based on the arming of the stem and the shape of the leaves are too inconstant to be used in establishing formal varieties, and unless more constant characters are found we must conclude that they do not exist in this species. We are of course dealing in such forms with groups of organisms very closely related to each other between which the differences are very slight. However small these differences may be, they must be constant, otherwise the supposed forms cease to exist and we have instead only a group of slightly dissimilar individuals.

This species occurs most abundantly on the lower dryer parts of the islands, but it is not uncommon to find it at elevations from 600 to 1,000 feet. It is well known that climatic conditions vary greatly within comparatively slight differences in elevations on these islands. The lower parts adjacent to the shore are very dry and support a xerophytic vegetation while the upper parts varying usually from 500 to 1,000 or more feet in elevation are moist with a mesophytic vegetation. Between these two there is usually an intermediate or transition region, covered with a vegetation which is made up for the most part of species from both the dry and moist region. Growing under such a variety of conditions as this we would naturally expect that the difference in environment would have a corresponding effect upon the habit and general appearance of this species, and such is the case. I believe, however, that the changes brought about by such causes in Castela are even less marked than in some of the other species of Galapagos plants which occur in like situations.

I wish to acknowledge the kindness of the authorities of the Gray Herbarium in sending me their series of specimens of this species for further study.

Department of Botany,
University of Wisconsin.

THE DEVELOPMENT OF PYRONEMA CONFLUENS VAR. INIGNEUM[1]

William H. Brown

In a preliminary note (Brown, '08) and in two subsequent papers (Brown, '10, '11) the writer has mentioned the variety named in the title as a form of *Pyronema confluens* Tul. in which the trichogyne did not fuse with the antheridium. This character and several minor ones seem, however, to distinguish it quite sharply from the ordinary form, and so it has been thought better to give it a varietal name. The one selected has reference to the conditions under which it grows.

A number of workers have quoted the writer as saying that there was no fusion of antheridium and trichogyne in *Pyronema confluens*, whereas in the note referred to (Brown, '08) it was distinctly stated that the form, there described, was probably derived from such a one as that observed by Harper ('00), in which this fusion did occur. The writer, as here described, has since observed this fusion in another form.

Culture of Pyronema

As the generic name implies, *Pyronema confluens* usually occurs on burnt places. Harper ('00) obtained the material with which he worked in abundance, on half charred masses of leaves; but also among damp, well decayed leaves which had not been burnt. He found the plant to be extremely hydrophytic, and unable to withstand the dryness of ordinary laboratory air for even a few hours. Harper notes the differences between the above conditions and those under which Kihlman obtained what Harper believes, from the similarity of morphological structures, to be the same species. Kihlman found his material in clefts on stones and obtained successive crops by watering the stones every three or four days. Kesaroff ('08) and Seaver ('09) found that Pyronema grew abundantly on soil sterilized by heat but not on unsterilized soil. Seaver's conclusions are of special interest here, since, through his kindness, the writer has been able to repeat his experiments with the same strain and to compare it with the variety inigneum. Seaver found that his strain always produced abundant crops on soil or leaf-mold which had been sterilized by heating to high temperatures or by steam heat if under sufficient pressure, while he was not able in a single case to produce more than a beginning of growth on unsterilized soil. There is nothing to indicate that Seaver found this strain to be hydrophytic and the writer has found that it grew very vigorously on the outside of clay flower pots which stood in an ordinary laboratory room where the air frequently became quite dry. The differences in the conditions of sterilization and moisture under which the strains, described by Harper, Kihlman, and Seaver, were grown would seem to indicate that in Pyronema, as in *Puccinia graminis* Pers. (Eriksson, '96), there may be different physiological varieties which are entirely similar morphologically. It is, of course, uncertain as to whether these strains are permanently different or could be changed from one to another by gradually changing the environmental conditions. There is, however, nothing to indicate that the latter is the case.

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[1] Botanical Contribution from the Johns Hopkins University, No. 44.

In view of the experiments of Van Tieghem ('84), who found that the course of development of Pyronema was markedly influenced by the external conditions, it seems altogether likely that the growth of this plant under different physiological conditions might produce different morphological varieties and this may have been the origin of the variety inigneum.

The variety inigneum was found about the first of April, 1908, growing on a flower-pot in the botanical laboratory of the Johns Hopkins University. The ascocarps were produced to some extent on the soil inside the pot, but were much more abundant on the pot itself, where they formed large patches on both the inner and the outer surfaces. The pot on which these were found had been used for growing fern prothalli and had been watered constantly since some time during the previous October. A large number of the pots used in the laboratory had been sterilized early in October but it is uncertain as to whether or not this particular one had been. It is certain, however, that since that time, it had not been subjected to any conditions approaching sterilization.

As soon as the fungus was found, the pot on which it was growing was transferred to another room and kept watered for the next two months, during which time ascocarps continued to be produced in large numbers. They were, however, never as abundant in subsequent crops as in the first one. This may have been due to differences in the two rooms or to some other factor; but it seems hardly likely that it was connected with conditions of sterilization, since the pot on which the fungus was growing had been kept moist for more than five months before the fungus appeared. This is more probable since Seaver ('08) found that keeping soil moist for a week destroyed the beneficial effects of sterilization. The pot was set aside early in June and allowed to dry. It was again watered about the first of the following October. Ascocarps were produced steadily during the next six weeks after which the fungus was attacked by small beetles and disappeared. It will be seen that the last ascocarps were produced more than twelve months after any possible sterilization.

During the fall of 1909 the form of *Pyronema confluens* described by Seaver ('09) was grown in the same room and on pots similar to those on which the variety inigneum had been grown the previous fall. In no case was more than a beginning of growth obtained on pots which had not been sterilized or which had been kept moist for more than a week after sterilization, while in every case in which the soil was moistened and inoculated, immediately after sterilization, ascocarps were produced in large numbers. These experiments, confirming as they do those performed by Seaver, seem to show that sterilization produced some change in the substratum which was essential for the growth of this strain. The growth of the variety inigneum seemed, however, to be quite independent of sterilization for it is hardly possible that any of the beneficial effects of sterilization should remain in a pot after twelve months, during eight of which it had been kept moist, thus allowing the growth of fungi and various micro-organisms. Although the two forms were grown at different times, the use of similar pots in the same room and at the same season of the year with such decidedly different results, in the two cases, would seem to show that the forms are quite distinct physiologically.

DEVELOPMENT

The ascocarps which were produced on the pots were formed on the surface of a mass of hyphae which frequently grew over a felt of Penicillium. Owing to this method of growth, ascocarps of all ages could be removed from the pot with a sharp scalpel without injury. That this was the case was shown by an absence of any signs of displacement or tearing in the specimens. The material was killed in medium chrom-acetic or Flemming's solution and stained with Heidenhain's haematoxylin or Flemming's triple stain.

For comparison with the variety inigneum the form of *Pyronema confluens* (omphalodes) described by Seaver ('09), which was grown as previously described, was prepared in the same way as the variety. In the figures of his strain, which in this discussion will be called the normal form, Seaver has figured trichogynes fused to the antheridia.

Owing to the many excellent descriptions and figures of *Pyronema confluens*, particularly the recent ones of Harper ('00) and Claussen ('12), it seems unnecessary here to more than point out the differences between the variety inigneum and the previous descriptions. The cytological features observed are very similar to those described by the writer (Brown, '11) for *Lachnea scutellata* (L.) Sacc. and present no new features.

The ascogonia of the variety inigneum are usually produced in rosettes of from fifteen to twenty. This is about twice the number in the normal form. De Bary ('84, p. 225), says that there are several, at least two or three, ascogonia in a rosette, while Harper ('00) figures three.

Many if not all of the ascogonia of a rosette of the variety inigneum are formed from the same hypha. The ascogonia are large, oval cells. Each is supported by a stalk, consisting of several cells which are smaller than the ascogonia, but larger than the other vegetative cells. The ascogonia and vegetative cells are all multinucleate. A long, slender, single-celled trichogyne is produced on the upper surface of each ascogonium. This trichogyne was found to differ from the form described by Harper, and from the normal form in lacking a beak or snout-like projection at its apex.

The antheridia are large, club-shaped, multinucleate cells and are quite similar to those figured by Harper ('00). The antheridia like the ascogonia are borne on stalks. In all cases in which the origin of an antheridium could be determined, it was found to be connected with the cell just below an ascogonium.

In the normal form, the trichogynes and antheridia become connected in the manner that has been described by Harper ('00), while it is at this point that the variety inigneum differs markedly from Harper's description. In the variety inigneum, the antheridia and ascogonia are much more independent of each other than in the normal form. Frequently an ascogonium and the antheridium connected with its stalk are so far apart that even if the trichogyne grew straight towards the antheridium its length would not be anything like great enough to connect the two. The growth of the trichogyne appears, moreover, to be quite independent of that of the antheridium. Frequently the trichogyne grew straight out in an opposite direction from that in which the antheridium developed. At other times, a trichogyne made several coils around an antheridium, while in some cases two trichogynes were coiled around the same antheridium. In only a few cases did the trichogyne grow up over the antheridium or direct its tip against the tip of the latter as described by Harper ('00). Owing to the extremely irregular behavior of the trichogyne, the few cases in which this did occur might as well be attributed to chance variation as to any attraction between antheridium and trichogyne. In no case was the fusion between trichogyne and antheridium observed. That the irregularity just described as well as the absence of fusion were not due to displacement in handling would seem to be shown by the opposite results obtained in the normal form. The evidence from sections is very convincing. According to Harper, after the trichogyne has fused with the antheridium the two remain permanently connected. In sections the ascogonia, trichogyne and antheridia of the variety inigneum retain their normal form long after the ascogenous hyphae have grown from the ascogonia. At this stage, the ascogonia, trichogyne and antheridia are all imbedded in a firm mass of vegetative hyphae, so that it would hardly seem possible that any displacement could occur without causing considerable tearing or other disturbances which would be plainly visible in the sections. These, however, showed no evidences of tearing or displacement except in rare cases in which young ascocarps were on the edges of the mass of material.

After an ascogonium has attained its mature form, the nuclei in the trichogyne degenerate in the manner described by Harper ('00). In the process the nucleoli enlarge while the nucleus swells up and the rest of its contents become transparent. The nucleoli are usually apparent for a short time after the nuclei go to pieces, while the remains of the nuclei produce a meshlike appearance in the cytoplasm. The antheridia retain their normal appearance long after the ascogenous hyphae have grown out from the ascogonia and sometimes they appear unchanged even after the formation of the first asci. After a time, however, their nuclei degenerate in the manner described for those in the trichogyne. After the nuclei degenerate, the antheridia become vacuolated and their contents degenerate. In the form described by Harper vacuoles appear in the antheridia after the nuclei have migrated into the trichogyne and at an earlier stage than in the variety inigneum. The behavior of the antheridia and their nuclei, as just described, would seem to be sufficient to show that the nuclei do not migrate into the trichogyne in the variety inigneum.

The development of the ascogenous hyphae is in accord with Harper's description. They grow out from the ascogonium as large branching hyphae, at the ends of which the asci are formed. The nuclei from the ascogonium migrate into these hyphae in an irregular manner and not in pairs as described by Claussen ('12). This early pairing of nuclei would not be expected in the variety inigneum for Claussen believes that each pair is composed of a nucleus derived from the ascogonium and one from the antheridium. A considerable proportion of the nuclei of the ascogonia never migrate into the ascogenous hyphae but degenerate in situ.

No fusion of nuclei, except of degenerating ones, was observed in the ascogonium or ascogenous hyphae, the only one observed being the usual one in the asci. There were, however, appearances resembling fusing nuclei. When the nucleus is preparing for division and is consequently large, the spireme or chromosomes frequently contract into a rather dense mass resembling a second nucleolus. The nucleus at this stage might readily be taken for a fusion nucleus. Also just after division, the daughter nuclei may reorganize so close to each other as to be pressed together and thus look like fusing nuclei. These phenomena have been described by the writer in detail in Lachnea (Brown, '11). The figures presented in the paper on Lachnea answer Claussen's criticism of the note on Pyronema.

The asci are formed from hook shaped hyphae at the tips of the ascogenous hyphae in the usual manner. Two nuclei divide simultaneously into four which become arranged in a uninucleate ultimate and antipenultimate cell and a binucleate penultimate one. The penultimate cell may then produce an ascus or a second hook. The ultimate and antipenultimate cells usually fuse, the nucleus of the antipenultimate one passing into the ultimate, which may then form another hook as figured by Claussen ('12) instead of degenerating as described by Harper ('00). This proliferation of the hooks, with an increase in the number of asci formed, has been described by Mc-

Cubbin ('10) for Helvella and by Brown ('10, '11) for Leotia, Lachnea and Geoglossum.

When a penultimate cell gives rise to an ascus, the two nuclei fuse, after which the fusion nucleus goes into synapsis. After synapsis a spireme emerges which contracts and divides into five chromosomes. These become arranged on the spindle and five daughter chromosomes pass to each pole. In doing so, they do not divide as in Lachnea (Brown, '11), but usually become dumb-bell shaped. In the next two divisions giving rise to eight spore nuclei, and in the first division of the spore nucleus, there are also five chromosomes. It thus appears that the same number of chromosomes, five, persists through all the divisions of the life history of the plant.

DISCUSSION

The fusion of the antheridium and trichogyne of *Pyronema confluens* was described by the Tulasne Brothers in 1866. Their description was confirmed by Kihlmann ('83), who regarded the fusion as a proof of the sexuality of this form.

Van Tieghem ('84), grew Pyronema under various conditions, and described the course of development as being markedly influenced by the different treatments. According to this writer, under certain conditions, the chief of which is the presence of sufficient moisture, the ascogonia and antheridia are large and the trichogynes fuse with the antheridia. Under other conditions, particularly a certain amount of dryness, the antheridia and ascogonia are smaller. Under still other conditions, when the dryness passes a certain limit or the temperature falls below a certain point, the cells which usually develop into ascogonia and antheridia are smaller and more numerous than in the other two cases and there is no differentiation into ascogonia and antheridia. Since development continued in all of these cases, Van Tieghem regarded the last as an argument against the sexuality of Pyronema but said that De Bary would regard it as a case of apogamy. Harper ('00) found that, after the fusion of the antheridium and trichogyne of Pyronema, the nuclei of the antheridium migrated into the trichogyne and then into the ascogonium. According to Harper this was followed by a fusion in pairs of the nuclei in the ascogonium. Dangeard ('05) described the fusion of the antheridium and trichogyne but said that the nuclei of the antheridium did not migrate into the ascogonium. Claussen ('07) confirmed Harper's account of the migration of the nuclei but denied the presence of a fusion of nuclei in the ascogonium. In the variety inigneum there appears to be neither a fusion of antheridium and trichogyne nor of nuclei in the ascogonium. Even though some of the above results may be due to misinterpretation it would still seem that *Pyronema confluens* may develop in a variety of ways. Some of the differences, such as those described by Van Tieghem, appear to be due to the influence of external conditions. Others, however, such as those described in this paper between the normal form and the variety inigneum may represent permanent varieties.

The absence of the fusion between the trichogyne and antheridium in some of Van Tieghem's cultures and in the variety inigneum prove that this is not necessary for the development of the ascocarp of Pyronema, while Claussen's results, together with the apparent absence of a fusion of nuclei in the ascogonia of the variety inigneum would seem to show that development can also occur without this nuclear fusion. These results, however, do not prove that a fusion of nuclei never occurs in the ascogonium. In view of the large number of pteridophytes in which apogamy has been induced, these results would not be very surprising even though it had been proved that in some cases a sexual fusion of nuclei did occur in the ascogonium. It may be said, however, that the development of Pyronema without a fusion of the antheridia and trichogynes or a fusion of nuclei in the ascogonium, together with the large number of cases among the Pezizineae in which different workers have failed to find a nuclear fusion in the ascogonium (see Brown, '11, and Claussen, '12), is not in favor of the view that such a fusion of nuclei does occur. Another argument against this fusion would seem to be afforded by the presence, in the ascogonia and in various other stages of the life history of the variety inigneum and of *Lachnea scutellata* (Brown, '11), of appearances, during the division of the nuclei, which simulate fusion quite closely. It is, of course, not safe to assume that conclusions drawn from one form will apply to another, but, as previously pointed out (Brown, '11), in view of the fact that in those Pezizineae in which divisions of the nuclei have been described in the ascogonia, these divisions show stages having the appearance of fusing nuclei, it would seem necessary to study the divisions in the ascogonia and to distinguish between true and apparent fusions before the occurrence of such a fusion can be regarded as proved, and, so far as the writer knows, divisions have not been seen in the ascogonia of any of the Pezizineae in which a fusion of nuclei has been described at this point.

SUMMARY

Pyronema confluens Tul. var. *inigneum* Brown agrees with the taxonomic description given by Saccardo for *Pyronema confluens* but differs from the ordinary form in that there is no fusion of antheridium and trichogyne.

The cultural conditions for the growth of the variety also appear to differ from those for the normal form in that sterilization of the substratum is unnecessary.

No fusion of nuclei was observed in the ascogonium or ascogenous hyphae. The only one observed occurred in the asci.

There are apparently five chromosomes in all of the nuclear divisions.

The work here reported was carried on at The Johns Hopkins University. The writer is indebted to Prof. D. S. Johnson for valuable suggestions and criticisms.

BUREAU OF SCIENCE,
MANILA, P. I.

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NEW OR NOTEWORTHY GRASSES*

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In a recent revision of the specimens of grasses from the United States in the U. S. National Herbarium, the following notes that seemed worthy of record were made.

MANISURIS L. Mant. Pl. **2**: 300. 1771. *Rottboellia* L. f. Nov. Gram. 23. 1779. *Hemarthria* R. Br. Prodr. Fl. Nov. Holl. 207. 1810. *Coelorhachis* Brogn. in Duperrey, Bot. Voy. Coquille 64. 1831. *Stegosia* Lour. Fl. Cochinch. 51. 1790.

We are considering these groups congeneric under the oldest name.

Manisuris fasciculata (Lam.). *Rottboellia fasciculata* Lam. Tabl. Encycl. **1**: 204. 1791. *Hemarthria fasciculata* Kunth, Rév. Gram. 1: 153. 1829.

RYTILIX GRANULARIS (L.) Skeels, U. S. Dept. Agr. Bur. Pl. Ind. Bull. 282: 20. 1913. *Cenchrus granularis* L. Mant. Pl. **2**: 575. 1771.

Skeels has shown that the earliest generic name for this species, hitherto referred to Manisuris and to Hackelochloa, is Rytilix Raf.

Andropogon stolonifer (Nash). *Schizachyrium stoloniferum* Nash in Small, Fl. Southeast. U. S. 59. 1903.

HOLCUS SORGHUM L. Sp. Pl. 1047. 1753.

HOLCUS HALEPENSIS L. loc. cit.

Elsewhere[1] have been indicated the reasons for using the name Holcus for the group of plants including the sorghums and Johnson grass. *Holcus sorghum* is the type of the genus Holcus. The grasses that have been included by recent authors under Holcus have received a new generic name Notholcus Nash.[2]

CHAETOCHLOA LUTESCENS (Weigel) Stuntz, U. S. Dept. Agr. Bur. Pl. Ind. Inv. Seeds 31: 36. 1914. *Panicum lutescens* Weigel, Obs. Bot. 20. 1772.

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[1] Contr. U. S. Nat. Herb. **12**: 195. 1909.

[2] Hitchcock in Jepson, Fl. Calif. 126. 1912. Nothoholcus Nash in Britt. & Brown, Illust. Fl. ed. 2. **1**: 214. 1913.

Stuntz has shown that the name *Panicum glaucum* L. should be applied to pearl millet, the grass that has been called *Pennisetum americanum* (L.) Schum. *Chaetochloa glauca* Scribn. is a typonym of this. The grass that has been called *C. glauca* by recent authors should be known as *C. lutescens*.

PENNISETUM GLAUCUM (L.) R. Br. Prodr. Fl. Nov. Holl. 195. 1810. *Panicum glaucum* L. Sp. Pl. 56. 1753.

As indicated above the name *Panicum glaucum* was applied by Linnaeus to the grass we call pearl millet, now referred to the genus Pennisetum as *P. spicatum*, *P. typhoideum* or more recently as *P. americanum* (L.) Schum. Robert Brown transferred *Panicum glaucum* to Pennisetum as *P. glaucum*. The plant he described was the species we have been calling *Chaetochloa glauca* but under the system of nomenclatorial types *Pennisetum glaucum* is a typonym of *Panicum glaucum* and the application of the name rests upon the identity of the latter species, independent of the species described when the transfer is made.

TORRESIA Ruiz & Pav. Syst. Veg. Peruv. Chil. 251. 1798. *Savastana* Schrank, Baier. Fl. 1: 100 & 337. 1789, not *Savastania* Scop. Introd. 213. 1777. *Hierochloë* R. Br. Prodr. Fl. Nov. Holl. 208. 1810.

Hierochloë R. Br. is conserved by the Vienna Code but the earlier name Savastana Schrank has been used for this genus by botanists working under the American Code. The latter name is, however, invalidated by the earlier homonym Savastania Scop. (Melastomaceae). Scopoli gives a generic description but mentions no species. However as he cites Tibouchina Aubl. the genus Savastania Scop. is properly published. The next name available is Torresia Ruiz & Pav. This name was first used in an earlier work,[3] but no species were given, so the name must date from 1798 where it is properly published with the type species *T. utriculata* Ruiz & Pav. The North American species are:

Torresia alpina (Swartz). *Holcus alpinus* Swartz; Willd. Sp. Pl. 4. 937. 1806.

Torresia macrophylla (Thurb.). *Hierochloë macrophylla* Thurb.; Boland. Trans. Calif. Agr. Soc. 1864–65: 132. 1866.

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[3] Ruiz & Pav. Fl. Peruv. Chil. Prodr. 125. 1794.

Torresia mexicana (Rupr.). *Ataxia mexicana* Rupr.; Fourn. Mex. Pl. 2: 71. 1886.

Torresia odorata (L.). *Holcus odoratus* L. Sp. Pl. 1048. 1753.

Torresia pauciflora (R. Br.). *Hierochloë pauciflora* R. Br. Suppl. App. Parry's Voy. 293. 1823. (Chloris Melvilliana.)

ARISTIDA ADSCENSIONIS L. Sp. Pl. 82. 1753. *A. humilis* H. B. K. Nov. Gen. & Sp. 1: 121. 1816. *A. bromoides* H. B. K. op. cit. 122.

The type of the earliest name is from the island of Ascension, the type of *A. humilis* is from Cumaná, Venezuela, the type of *A. bromoides* from the equatorial Andes. The three names appear to apply to the same species, which ranges throughout tropical America and in the warmer parts of the Old World.

Stipa pulchra n. sp.

A cespitose perennial; culms scaberulous or smooth, pubescent below the nodes, mostly 60 to 100 cm. high; sheaths smooth or scaberulous; ligule truncate, 2 to 3 mm. long, or shorter on the innovations; blades flat or soon involute, 1 to 4 mm. wide, pilose above, scaberulous beneath; panicle open, 10 to 30 cm. long, the main axis smooth or scaberulous, the branches slender, scaberulous, ascending or spreading, somewhat flexuous, mostly in pairs, naked below, the lower 8 to 15 cm. long, sometimes pubescent around the axils; spikelets loosely clustered toward the ends of the branches, the branchlets slender, the ultimate lateral pedicels 2 to 3 mm. long; glumes nearly equal, usually purple, attenuate-pointed, about 15 mm. long, the lower 3-nerved, the upper 5-nerved; lemma oblong, including the narrow sharp pilose callus 8 to 10 mm. long, pubescent in lines from below to about the middle or somewhat pubescent all over, the surface minutely tuberculate, the apex somewhat constricted into a neck, the edge of this ciliate with erect hairs; awn 6 to 8 cm. long, twice geniculate, appressed pilose to the first bend, scabrous above, the terminal segment slender and flexuous.

Type specimen in the U. S. National Herbarium, no. 416590, collected in a railroad cut three miles south of Healdsburg, Sonoma County, California, April 9, 1902, by A. A. Heller (no. 5252). Common in California throughout the state at lower altitudes. This species has been referred to *S. setigera* Presl.[4] The latter differs in having a fruit 6 mm. long with a well-marked neck, with a crown merely toothed and not long-ciliate, and with an awn only about 5 cm.

[4] Rel. Haenk. 1: 226. 1830.

long. The origin of Presl's type is not known. It does not quite agree with any North American species. The above notes were taken on the type in the Bohemian National Museum at Prague.

Kneucker[5] states that the species that has been called *Stipa setigera* is *S. tenuis* Philippi. I have examined a duplicate type specimen of *S. tenuis* and find that it differs from *S. pulchra* in several important respects. The glumes are shorter and the awns are capillary, flexuous, but not geniculate.

Stipa lepida n. sp.

A cespitose perennial; culms erect, smooth or scaberulous, pubescent below the nodes, 50 to 80 cm. high; sheaths smooth or scaberulous, or sometimes a little pubescent, more or less villous at the mouth; ligule a narrow membrane about 0.5 mm. long; blades flat, more or less involute in drying, 10 to 30 cm. long, 1 to 4 mm. wide, pubescent above, smooth or scaberulous beneath; panicle loose, 10 to 13 cm. long, the axis smooth or scaberulous, the branches single or in pairs, or the lower sometimes in threes, spreading, scabrous, slender, naked below, sometimes pilose in the lower axils, the lower nodes distant; spikelets pale or purplish, clustered on the upper half or two thirds of the branches, the branchlets appressed; glumes thin, narrow, gradually acuminate, slightly unequal, the lower 7 mm. long, 3-nerved, the upper 3-nerved or faintly 5-nerved; lemma about 5-nerved, pilose on the callus, rather sparsely pubescent all over or glabrate above, narrowed toward the apex but with no distinct neck, the inconspicuous crown minutely ciliate; awn mostly 2.5 to 3.5 cm. long, very slender, minutely appressed pubescent below or nearly glabrous, scabrous above, twice geniculate, the bends often indistinct, the terminal segment somewhat flexuous.

Type specimen in the U. S. National Herbarium, no. 733683, collected on an open hillside in the Santa Ynez Forest Reserve, Santa Barbara County, California, April 19, 1910, by Agnes Chase (no. 5611).

This species, common in California at lower altitudes, has been referred to *Stipa eminens* Cav.[6] The latter, the type of which was examined at the herbarium of the Botanical Garden at Madrid, differs in having long ligules, the uppermost as much as 3 mm. long, and in having 3 or 4 more flexuous branches at each node of the panicle, and a more flexuous awn. A synonym is *Stipa flexuosa* Vasey.[7] *Stipa eminens* is found on the highlands of Mexico from Puebla to Sonora and extends into the United States from southern Arizona to western Texas.

[5] Allg. Bot. Zeitschr. **19**: 171. 1913.

[6] Icon. Pl. **5**: 42, pl. 467. f. 1. 1799.

[7] Bull. Torrey Club **15**: 49. 1888.

Stipa lepida Andersoni (Vasey). *Stipa eminens Andersoni* Vasey, Contr. U. S. Nat. Herb. 3: 54. 1892.

This differs in being smaller, and in having narrower and fewer-flowered panicles and somewhat smaller spikelets. The type was collected by Anderson near Santa Cruz, California. The published type locality, "Lower California," is an error.

MUHLENBERGIA TRIFIDA Hack. Repert. Nov. Sp. Fedde **8**: 518. 1910.

This is the common western grass hitherto known as *M. gracilis*, which name is a synonym of *M. quadridentata* (H. B. K.) Kunth.

MUHLENBERGIA EMERSLEYI Vasey, Contr. U. S. Nat. Herb. 3: 66. 1892. *M. Vaseyana* Scribn. Rep. Mo. Bot. Gard. 10: 52. 1899.

This species has been confused with *M. distichophylla* Presl which is confined to Mexico. This group of Muhlenbergia is more nearly related to Epicampes than to most of the other species of Muhlenbergia.

Sporobolus contractus new name. *Sporobolus strictus* (Scribn.) Merr., U. S. Dept. Agr. Div. Agrost. Circ. 32: 6. 1901, not *S. strictus* Franch. Bull. Soc. Hist. Nat. Autun 8: 368. 1893.

Sporobolus macrus (Trin.). *Vilfa macra* Trin. Mém. Acad. St. Pétersb. VI. Sci. Nat. 4^1: 79. 1840.

This species, hitherto known only from the type specimen and unrecognized in our flora, is allied to *Sporobolus Drummondii* (Trin.) Vasey, but differs from all the species of this group in having creeping scaly rhizomes. The type of *Vilfa macra* has been examined at the herbarium of the St. Petersburg Academy of Sciences. The type locality as published and as given on the label is "Louisiana," without other data. The only other specimen known to us was collected in wet pine land near Biloxi, Mississippi, October 8, 1907, by Agnes Chase (no. 4341).

AGROSTIS EXIGUA Thurb. in S. Wats. Bot. Calif. **2**: 275. 1880.

This very rare species has been known for several years only from the single specimen in the Gray Herbarium collected by Bolander in the "foothills of the Sierras." Specimens of this species have been recently sent in from Howell Mountain, Napa County, California, collected by Mr. Joseph P. Tracy (no. 1552).

Agrostis exarata ampla (Hitchc.). *Agrostis ampla* Hitchc. U. S. Dept. Agr. Bur. Pl. Ind. Bull. 68: 38. 1905.

Agrostis exarata microphylla (Steud.). *Agrostis microphylla* Steud. Syn. Pl. Glum. **1**: 164. 1854.

It seems more in accord with the facts to consider *Agrostis exarata* to be a polymorphous or variable species including the above mentioned forms as varieties. The United States specimens referred to *Agrostis glomerata* (Presl) Kunth[8] appear to be better placed under *A. exarata.*

AGROSTIS SCHIEDEANA Trin. Mém. Acad. St. Pétersb. VI. Sci. Nat. 4^1: 327. 1841.

The type of this comes from Mexico. The specimens from the United States that have been referred to this species[9] prove to belong to *A. oregonensis* Vasey. The type of *A. Schiedeana* belongs to *A. perennans.* The specimens cited under *A. Schiedeana* in Mexican Grasses,[10] all from the plateau of Mexico, belong to *A. Bourgaei* Fourn., a valid species.

CALAMAGROSTIS SCABRA Presl, Rel. Haenk. **1**: 234. 1830.

This species has been included under *C. Langsdorfii* Trin. (Gram. Unifl. 225. 1824), which differs in having longer callus hairs and less scabrous glumes. The latter is referred by Hackel to *C. villosa* Mutel, as a variety (*C. Halleriana Langsdorfii* Hack. in Sommier, Nuov. Giorn. Bot. Ital. **25**: 98. 1893; *C. villosa Langsdorfii* Hack. Bull. Herb. Boiss. **7**: 650. 1899). *Calamagrostis scabra* is closely related to *C. canadensis* (Michx.) Beauv. and may be only a form or variety of that species. The former appears to be confined to the vicinity of the coast of Alaska and southward while the latter is the dominant grass of the interior of Alaska. Presl's species was first described from Nutka Sound, Vancouver Island.

Notholcus mollis (L.). *Holcus mollis* L. Syst. Nat. ed. 10. 2: 1305. 1759.

This species not previously reported from the United States has been sent to the U. S. National Herbarium from Eureka, California, by Mr. Joseph P. Tracy. It differs from *N. lanatus,* common on the Pacific Coast, in having creeping rhizomes and glabrous sheaths.

Sphenopholis pennsylvanica (L.). *Avena pennsylvanica* L. Sp. Pl. 79. 1753.

Scribner[11] in his article on Sphenopholis takes up Michaux's name and calls the species *Sphenopholis palustris* (Michx.) Scribn. He ignores *Avena pennsylvanica* L. because Linnaeus described the species as having a villous ovary ("seminibus villosis"). The specimen in the Linnaean Herbarium collected by Kalm has been examined and it is identical with the species that has been called *Sphenopholis palustris* and *Trisetum pennsylvanicum* Beauv. The ovary is not pubescent but Linnaeus's description applies in other respects. As the name is based on a specimen collected in Pennsylvania by Kalm, we may assume that this specimen is the type and that the description is in error as to the statement "seminibus villosis."

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[8] See North American Species of Agrostis, U. S. Dept. Agr. Bur. Pl. Ind. Bull. 68: 30. 1905.

[9] See North American Species of Agrostis, op. cit.

[10] Contr. U. S. Nat. Herb. **17**: 319. 1913.

[11] Rhodora **8**: 137–146. 1906.

Danthonia Cusickii (Williams). *Danthonia intermedia Cusickii* Williams, U. S. Dept. Agr. Div. Agrost. Circ. 30: 7. 1901.

This differs from *D. intermedia* Vasey in being more robust, with larger spikelets, and with acute or acuminate rather than aristate lobes of the lemma.

Danthonia Macounii n. sp.

A cespitose perennial; culms erect, glabrous, 30 to 50 cm. high; sheaths villous, the hairs from small papillae; ligule a narrow ciliate-margined membrane; blades flat, becoming involute, villous above, glabrous beneath, erect, 10 to 20 cm. long, 3 to 4 mm. wide, the basal flexuous and usually narrower; panicle narrow, 4 to 6 cm. long, the branches scabrous-pubescent, appressed; spikelets 5- to 6-flowered; glumes equal, longer than the florets, faintly several-nerved, gradually acuminate, smooth except the slightly scabrous apex, about 15 mm. long; lemma villous on the rounded back, about 5 mm. long to the base of the awn, the lobes about 5 mm. long, abruptly narrowed into slender awns; awn of lemma about 10 mm. long, flat, loosely twisted in about 2 coils and brown at base, the upper portion slender, straight, divaricate.

The type specimen in the U. S. National Herbarium, no. 733685, collected in the vicinity of Nanaimo, Vancouver Island, July 4, 1908, by John Macoun (no. 78825).

Other specimens in the U. S. National Herbarium are: Vancouver Island, Nanaimo, Macoun 39 in 1887, Summit of Mt. Benson, Macoun 78823; Chilliwick Valley, B. C., Macoun 26080.

This species resembles *Danthonia intermedia* Vasey in habit and inflorescence but differs in the villous lemma and the aristate teeth of the lobes. From *D. thermale* Scribn. it differs in its larger spikelets and villous sheaths.

Campulosus floridanus n. sp.

A perennial with short creeping scaly rhizomes; culms erect, puberulent or scaberulous below the inflorescence, otherwise glabrous, 60 to 100 cm. high; sheaths glabrous, the basal numerous, becoming fibrous with age; ligule a more or less lacerate membrane, the upper acute, 2 mm. long, the lower shorter; blades firm, mostly becoming involute, attenuate at apex, glabrous, 10 to 15 cm. long, the uppermost shorter, 1 to 3 mm. wide; spike long-exserted, single, erect, straight or somewhat curved, often twisted, 8 to 15 cm. long, the outer side of the rachis smooth and rounded, the margins somewhat incurved; spikelets densely imbricated, pectinate, consisting of 2 glumes, 2 sterile lemmas, a fertile floret and 1 or 2 upper reduced and sterile florets; glumes unequal, the first lanceolate, acuminate, 1-nerved, thin, 2 mm. long, the second firm, acuminate, ridged with 2 nerves, the third nerve weak, smooth or scaberulous on the nerves, about 6 mm. long, the awn slightly below the middle, straight and divaricate at maturity, about 5 mm. long, tuberculate at base; first sterile lemma about 3.5 mm. long, long-villous on the base and margins, bifid at apex, with a straight awn from between the lobes, reaching about the apex of the fertile floret, the palea wanting; second lemma similar to the first, but with longer awn and villous chiefly above the middle, the palea well-developed; fertile lemma about 5 mm. long, sparsely villous above the middle, awnless, the palea nearly as long; reduced floret not as long as the spikelet, sometimes with a second smaller rudiment above.

Type specimen in the U. S. National Herbarium, no. 726521, collected in East Florida, in 1875, by A. H. Curtiss.

Sandy pine woods, Florida. Other specimens in the U. S. National Herbarium are: Lake City, Nash 2212; Waldo, Combs 698, 727; Gainesville, Combs 761; Duval County, Fredholm 313.

This species has been referred to *C. chapadensis* Trin. of Brazil. That differs in having scabrous sheaths and blades and usually 2 flexuous spikes, in the tuberculate second glume roughened on the midnerve, and in the more densely villous florets.

Gymnopogon Chapmanianus n. sp.

A cespitose somewhat purple perennial; culms erect or stiffly ascending, 25 to 40 cm. high; leaves mostly towards the lower part of the culm; sheaths glabrous, striate, more or less inflated, strongly overlapping; ligule a very narrow ciliate-margined membrane; blades flat, smooth beneath, scaberulous above, scabrous on the margins, rather leathery, stiffly spreading, abruptly narrowed or somewhat cordate at base, pungently pointed, 4 to 7 cm. long, 4 to 6 mm. wide; panicle fan-shaped, consisting of numerous slender, straight, ascending branches about 15 cm. long, arranged along a sulcate, scabrous axis about 10 cm. in length, floriferous to the base; spikelets nearly sessile along the branches, somewhat remote but regularly arranged, usually not reaching the one above and opposite, 2- to 3-flowered, or more rarely 4-flowered, the florets distant and zig-zag on a slender rachilla; glumes narrow, attenuate-pointed, 1-nerved, smooth, scabrous on the keel, 3 to 3.5 mm. long, as long as or longer than the florets; lemmas somewhat compressed, scarcely keeled, 3-nerved, the lateral nerves faint, minutely and rather sparsely pilose, more densely pilose on the callus and on the inrolled margin, about 2 mm. long, the apex slightly bifid, the midnerve of the lower floret extending as a straight slender awn 1 to 2 mm. long; rachilla usually extending above the base of the uppermost floret.

Type specimen in the U. S. National Herbarium, no. 733684, collected in a sandy meadow at Sanford, Florida, September 25, 1907, by Agnes Chase (no. 4135).

Confined to central peninsular Florida. Other specimens in the National Herbarium are: Sanford, Chase 4134; Titusville, Chase 3999; Brevard County, Drawdy 6062; Bartow, Combs 1186; Tampa, Combs 1356; Braidentown, Combs 1334, Tracy 7102; Manatee, Chapman in 1886.

This species is distinguished from *G. ambiguus* by the ascending spikes and the shorter awns and from *G. brevifolius* by the ascending spikes that are evenly floriferous to the base. On the Chapman specimen above mentioned is a note by Dr. Chapman which says, "If we have *two* species [of Gymnopogon] then we must have *three* for I have one with 2–4 perfect flowers in the spikelet."

WILLKOMMIA TEXANA Hitchc. Bot. Gaz. 35: 283. 1903.

At the time this species was published it was stated that the name Willkommia had been applied to a group of Compositae before it was used by Hackel for a genus of grasses. Under the American Code as revised this earlier use does not invalidate Willkommia Hack. Willkommia Schultz-Bip. was mentioned only as a synonym of a species of Senecio[12] and hence was not properly or technically published.

The following specimens, all from Texas, are now in the U. S. National Herbarium: Ennis, J. G. Smith in 1897; Beeville, Smith in 1897; Kingville, Tracy 8903; near Houston, Thurow 10; Waller, Thurow 11 in 1906; Harvester, open "hard-pan" spot, Hitchcock 1205 in 1906.

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[12] Nyman, Consp. Fl. Eur. 357. 1879.

PAPPOPHORUM BICOLOR Fourn. Mex. Pl. **2**: 133. 1886.

This Mexican species, rather common in southern and western Texas, is to be added to our list of the grasses of the United States.

ORCUTTIA CALIFORNICA Vasey, Bull. Torrey Club 13: 219. pl. 60. 1886.

This species, known previously only from the type collection, from near San Quentin Bay, Lower California, has been recently collected in Goose Valley, California, by Miss Alice Eastwood (no. 1013). The type specimen was rather immature. Miss Eastwood's specimens are well-developed and show an elongated inflorescence 6 to 8 cm. long, with somewhat the aspect of *Lolium multiflorum*.

ERAGROSTIS BARRELIERI Daveau, Journ. de Bot. **8**: 289. 1894.

This species from the Mediterranean region is well established in Texas. It is allied to *Eragrostis Eragrostis* (L.) Karst. (*E. minor* Host) from which it differs in the linear many-flowered divergent spikelets and in the absence of glands on the foliage. The following specimens, all from Texas, are in the U. S. National Herbarium: Abilene, Tracy 7917; Kerrville, Heller 1879, Hitchcock 5280, J. G. Smith in 1897; Llano, Smith in 1897; San Antonio, Hitchcock 5164; Kenedy, Hitchcock 5341; Brownsville, Hitchcock 5428.

Eragrostis floridana n. sp.

A cespitose perennial; culms erect from a usually spreading base, smooth, 20 to 50 cm. high; leaves mostly basal, the cauline 1 or 2; sheaths shorter than the internodes, villous or the uppermost nearly smooth, a prominent tuft of hairs at the apex; ligule a narrow densely ciliate ring with numerous long hairs at the base of the blade; blades flat or soon involute, villous, especially beneath, 5 to 15 cm. long, 1 to 3 mm. wide; panicle finally exserted, open, 10 to 20 cm. long, nearly as wide, the branches stiffly spreading, finally horizontal or the lower reflexed, mostly 1 to 3 below, single above, villous in the axils, the main rachis smooth or scaberulous above, the branchlets and pedicels slender, flexuous, scabrous, the pedicels mostly as long as or longer than the spikelets, spreading; spikelets greenish or lead color, ovate-lanceolate or oblong, 3 to 4 mm. long, 1 to 1.5 mm. wide, mostly 5- or 6-flowered, the florets slightly imbricate; glumes ovate, acute, unequal, scaberulous on the keel above, the first about 1 mm. long; lemmas acutish, scarcely keeled, faintly 3-nerved, smooth, 1.5 mm. long; palea persistent, scabrous on the keels.

Type in the U. S. National Herbarium, no. 726520, collected in dry pine woods near Tampa, Florida, March, by A. H. Curtiss (no. 3494*).

This was distributed in the exsiccatae of North American Plants as *Eragrostis lugens* Nees.

Other specimens in the U. S. National Herbarium, all from dry sandy pine woods in western peninsular Florida, are: Tampa, Curtiss C in 1886, Combs 1342; Lakeland, Hitchcock 841. What appears to be the same species comes from the vicinity of Mt. Orizaba, Mexico, Bourgeau 2643. Another specimen of the same collected in Mexico by Botteri, but without definite locality, may come from the same region.

This species resembles *E. lugens* Nees, which differs in having smooth foliage, longer spikelets and longer and more acute lemmas. *Eragrostis flaccida* Lindm., from southern Brazil, is more delicate, with smaller and more slender spikelets.

ERAGROSTIS CILIANENSIS (All.) Link.

This name replaces *Eragrostis megastachya* (Koel.) Link.[13]

Poa Merrillana. *Poa glacialis* Scribn. & Merr. Contr. U. S. Nat. Herb. 13: 68. 1910, not Stapf, Journ. Linn. Soc. Bot. **37**: 532. 1906.

Poa Wrightii (Scribn. & Merr.). *Colpodium Wrightii* Scribn. & Merr. Contr. U. S. Nat. Herb. 13: 74. 1910.

Panicularia erecta (Hitchc.). *Glyceria erecta* Hitchc. in Jepson, Fl. Calif. 1: 161. 1912.

Agropyron sericeum n. sp.

Plants tufted, without rhizomes; culms smooth, erect, 60 to 100 cm. high, the nodes glabrous; sheaths glabrous, striate; ligule membranaceous, about 1 mm. long; blades flat, scabrous above, slightly scabrous beneath, 12 to 20 cm. long, 5 to 10 mm. wide, the auricles minute; spikes long-exserted, slender, erect, 10 to 15 cm. long, the rachis hispid-scabrous on the margins, the spikelets somewhat distant, exposing the rachis, not reaching the one above on the same side; spikelets appressed, 10 to 20 mm. long, green or sometimes purplish, 3- to 6-flowered; glumes 5 to 10 mm. long, about half as long as the spikelet, broad, abruptly acute, glabrous or sometimes villous near the base, distinctly 3-nerved, with sometimes 1 or 2 pairs of secondary nerves, the margin scarious, the nerves more or less scabrous; lemmas 10 to 12 mm. long, obscurely nerved below, distinctly 5-nerved above, short-villous except the glabrous tip, acute, usually extending into an awn 1 to 2 mm. long; palea hispid-ciliate on the keels.

Type in the U. S. National Herbarium, no. 725185, collected on alluvial soil along the Yukon River at Dawson, Yukon Territory, July 19, 1909, by A. S. Hitchcock (no. 4389).

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[13] See Hubbard, Philipp. Journ. Sci. Bot. **8**: 159–161. 1913.

Alluvial soil, Seward Peninsula and Yukon Valley.

Represented in the U. S. National Herbarium by 29 specimens from various localities from the White River to Fairbanks, the Koyukuk River and Anvik; also at Nome (Hitchcock 4809).

U. S. Department of Agriculture,
Washington, D. C.

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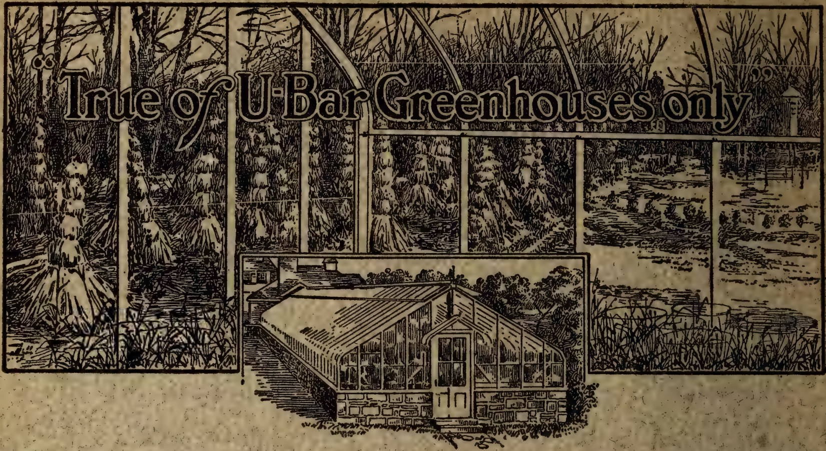
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THE EXCHANGE OF IONS BETWEEN THE ROOTS OF LUPINUS ALBUS AND CULTURE SOLUTIONS CONTAINING TWO NUTRIENT SALTS[1]

Rodney H. True and Harley Harris Bartlett

It has been shown by the authors in a former paper[2] that in unmixed dilute solutions of the common nutrient salts seedlings of *Lupinus albus* respond differently to different salts with respect to absorption. Calcium salts are absorbed from solutions of all dilutions tested, as indicated by the clearly marked decrease in the electrical conductivity of these solutions during the life of the seedlings grown in them. Magnesium salts are absorbed in a slight degree and only from solutions of a markedly limited range of concentration. Potassium salts either behave like distilled water and cause a loss of electrolytes at all dilutions, as in the cases of KH_2PO_4 and KCl, or permit only a minimal absorption phase to come to expression in the more favorable concentrations, as in solutions of K_2SO_4 and KNO_3. It seems that a predominating influence is exerted by the kations; calcium, magnesium or potassium action being clearly marked whatever the accompanying anion may be. The anions, however, are not without significance in relation to absorption.

In this investigation the same test plant, *Lupinus albus* L., was exposed to solutions containing mixtures of the nitrates of Ca, Mg and K *in pairs* in different proportions and in different total concentrations. The more concentrated members of the series approached in total salt content the soil solution as it is likely to be found in the vicinity of

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[1] Published by permission of the Secretary of Agriculture.

[2] True, R. H., and Bartlett, H. H. The exchange of ions between the roots of Lupinus albus and culture solutions containing one nutrient salt. Amer. Journ. Bot. **2**: 255–278. 1915.

Washington, D. C. This paper therefore deals primarily with the physiological interactions sometimes called antagonisms,[3] as these come to expression in modifying root absorption. It was intended by the use of mixtures of the same salt of several bases to get a comparative expression of the effect of the different combinations of kations concerned.

METHODS

The methods used have been described elsewhere,[2, 4] and the reader is referred to the details there given. The sole change involved in the investigation reported in the present paper lies in the fact that the solutions contained mixtures of salts in pairs.

EXPERIMENTAL RESULTS

Calcium Nitrate and Potassium Nitrate. Experiment 1

It was proposed in this experiment to ascertain the effect of calcium and potassium ions in the presence of the common anion, NO_3^-, on the absorption or excretion of electrolytes.

Four groups of solutions were prepared, each of which consisted of five chemically equivalent solutions, grading by fourths from pure $Ca(NO_3)_2$ to pure KNO_3, as follows:

1. 4/4 $Ca(NO_3)_2$. Ratio of kations to anions, 4 Ca^{++} : 8 NO_3^-.
2. 3/4 $Ca(NO_3)_2$ + 1/4 KNO_3. Ratio of kations to anions, 3 Ca^{++} + 2K^+ : 8 NO_3^-.
3. 2/4 $Ca(NO_3)_2$ + 2/4 KNO_3. Ratio of kations to anions, 2 Ca^{++} + 4 K^+ : 8 NO_3^-.
4. 1/4 $Ca(NO_3)_2$ + 3/4 KNO_3. Ratio of kations to anions, 1Ca^{++} + 6 K^+ : 8 NO_3^-.
5. 4/4 KNO_3. Ratio of kations to anions, 8 K^+ : 8 NO_3^-.

Thus, each member of a group had the same NO_3^- concentration and approximately the same electrical conductivity. Disregarding valence, the ratios of kations to anions in the five solutions of each group formed the series 4 : 8, 5 : 8, 6 : 8, 7 : 8, and 8 : 8. The four groups themselves formed a series ranging by intervals of $120\ N \times 10^{-6}$ from 120 to $480\ N \times 10^{-6}$. In addition to the twenty salt solutions, there was a check culture in distilled water.

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[3] True, R. H. Antagonism and Balanced Solutions. Science, n. s. 41: 653–656. 1915.

[4] True, R. H., and Bartlett, H. H. Absorption and Excretion of Salts by Roots, as Influenced by Concentration and Composition of Culture Solutions. I. Concentration Relations of Dilute Solutions of Calcium and Magnesium Nitrates to Pea Roots. U. S. Department of Agriculture, Bureau of Plant Industry, Bull. 231, 1912.

In figure 1 the daily concentration of each member of the four groups of solutions is shown for a total experimental period of 16 days. On the horizontal axis the unit is one day. The original total salt concentration of any solution in terms of gram equivalents per million liters is given at the left on the perpendicular axis. An increase in concentration of a solution during the experiment is therefore indicated by an upward trend of the curves; a decrease by the converse. The changes in concentration of the five solutions belonging to each group are of course indicated by a family of curves departing from the same point on the axis of ordinates. The course of the concentration changes taking place in the distilled water check is indicated by a curve starting at 0 on the axis of ordinates and covering about 12 days prior to the onset of changes attributed to exhaustion of the plants.

In the $120\ N \times 10^{-6}$ group of solutions all members seemed to gain ions during the first two days. This apparent loss of electrolytes

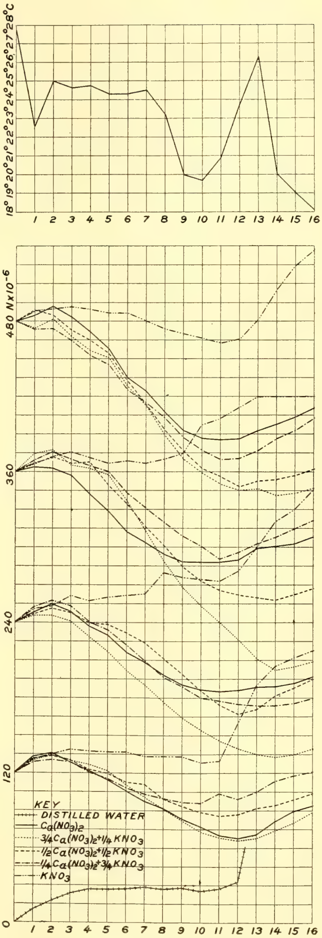

FIG. 1. Curves showing the change in concentration of the $Ca(NO_3)_2$ and KNO_3 solutions used in experiment 1.

on the part of the plants was probably due in a large measure to the gradual saturation of the solution with CO_2 from the living roots rather than to the loss of salts.[5] The plants in the KNO_3 solution lost electrolytes at a rather regular rate until the 11th day when probably through exhaustion or injury the roots began to give up ions rapidly, a course continued during the five remaining days of the experimental period. This course was closely paralleled by the plants grown in the control culture in distilled water. The plants in the $Ca(NO_3)_2$ solution began to absorb fairly rapidly on the third day and continued to do so at a relatively uniform rate for the next ten days. At that turning point, the plants began to give up ions more rapidly than they absorbed them and continued to do so at a relative uniform rate until the close of the experiment. All mixtures in this group were more favorable for absorption than KNO_3 alone. When the solution contained $3/4\ Ca(NO_3)_2 + 1/4\ KNO_3$ the result differed but little from that seen in the pure $Ca(NO_3)_2$ solution. In the solution containing $2/4\ Ca(NO_3)_2 + 2/4\ KNO_3$ a clear decrease in absorption took place. When $1/4\ Ca(NO_3)_2 + 3/4\ KNO_3$ was offered the favorable action of the small quantity of Ca salt so geatly outweighed in its influence the larger quantity of KNO_3 present that a marked net absorption was seen although it was less than in all other mixtures of this total concentration. The most favorable mixture was that containing $3/4\ Ca(NO_3)_2 + 1/4\ KNO_3$.

In the group having a total concentration of $240\ N \times 10^{-6}$ a very similar situation was found in the solutions of the pure salts. In KNO_3 the plants lost electrolytes at all stages of the experiment, rather slowly and regularly for the first week, more rapidly in the immediately succeeding days and very rapidly after the 11th day. In respect to absorption the KNO_3 solution was obviously again in the same class as distilled water. In the $Ca(NO_3)_2$ solution, after the usual preliminary period of stand-still, absorption set in and continued to the end of the experiment, a somewhat greater total absorption being seen than in the weaker solution of the same salt in the $120\ N \times 10^{-6}$ group. In the mixed solutions the favorable influence of combining calcium with potassium was strikingly seen. In all proportions of these kations the excess of absorption over loss was greater after the 9th day than in the pure calcium nitrate solution, although potassium nitrate in a pure condition was itself detrimental to the absorption process. The greatest net absorption was seen after the 13th day in the mixture containing 3/4 $Ca(NO_3)_2$ + 1/4 KNO_3, from which the total amount of ions absorbed was approximately double that taken out of 4/4 $Ca(NO_3)_2$ solution. Absorption from the solution containing equal proportions of the two salts lagged slightly behind that from the full calcium solution during the first ten days but during the succeeding six days exceeded it. A somewhat similar record was made in the solution containing 1/4 $Ca(NO_3)_2$ + 3/4 KNO_3. The plants in this mixture absorbed for the first ten days practically as they did in the full $Ca(NO_3)_2$ solution but showed a marked gain in the latter part of the period. Although the roots could not maintain a balance in favor of absorption when grown in a solution of pure KNO_3, nevertheless this salt greatly facilitated absorption when it was mixed with $Ca(NO_3)_2$. It also appears clear that a small proportion of $Ca(NO_3)_2$ is able to give to a mixture containing a large proportion of KNO_3 such properties as enable the roots to maintain active absorption. In the 240 $N \times 10^{-6}$ group of solutions the mutually helpful influence of Ca and K ions in respect to absorption comes sharply to the front.

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[5] For a full discussion of this point see Amer. Jour. Bot. **2**: 259. 1915.

In the third group of solutions each member had a total salt concentration of 360 $N \times 10^{-6}$. The effects on root absorption were in general like those seen in the last group. The increase of total salt content, however, seemed to cause an increased absorption of ions from all solutions. Although in the KNO_3 solution a net gain in ions by the plant was not indicated at any time, there was an absorption phase between the third and eighth days, succeeded by the usual period of loss during the remainder of the experiment. In the 4/4 $Ca(NO_3)_2$ solution root absorption was greater than in the less concentrated solutions of this salt. The maximum reduction in the concentration of the solution, attained during the period from the ninth to the twelfth day, was over 72 $N \times 10^{-6}$. The greatly increased absorption in the mixture containing 3/4 $Ca(NO_3)_2$ to 1/4 KNO_3 was very striking, the maximum quantity of electrolytes gained by the plants prior to the fourteenth day being indicated by a change of about 158 $N \times 10^{-6}$ in the concentration of the culture solution, *i. e.*, over double that gained from the pure solution of $Ca(NO_3)_2$. In the solution containing 2/4 $Ca(NO_3)_2$ + 2/4 KNO_3 the maximum absorption was reached about the fourteenth day and caused a reduction of about 102 $N \times 10^{-6}$ in the concentration of the culture solution, a markedly greater absorption than that from the 4/4 $Ca(NO_3)_2$ solution.

From the solution containing 1/4 $Ca(NO_3)_2$ + 3/4 KNO_3 there was somewhat less absorption than from the 4/4 $Ca(NO_3)_2$ solution.

In the group of strongest solutions, with a total salt concentration of 480 N × 10^{-6}, a further general increase in the rate of absorption was seen. Absorption in the mixtures increased as the calcium content increased, all being more favorable for absorption than the check in 4/4 $Ca(NO_3)_2$ solution. A clearly marked absorption phase was seen for the first time in the solution of KNO_3. This was probably due to the concentration factor, as has been indicated in a former study[6] of absorption in KNO_3 solutions.

The most striking results of this experiment may be summarized briefly as follows:

1. Root absorption from dilute solutions of $Ca(NO_3)_2$, of KNO_3, and of mixtures of these in a graded series increased in general with the increase of concentration from 120 to 480 N × 10^{-6}. Except in the highest concentration tested the plants lost more electrolytes in KNO_3 solutions than they absorbed and in the highest concentration the net gain of ions was small. Calcium nitrate in all concentrations supported an active absorption. It seems clear that in the series of concentrations here studied the ratio of Ca ions to K ions most favorable to the process of absorption is 3 Ca^{++} to 2 K^+ (3/4 $Ca(NO_3)_2$ + 1/4 KNO_3). In every group except that having the lowest total concentration there was greater absorption from mixtures in all proportions than from solutions of either of the component salts in a pure state.

In the solutions having the lowest concentration, it is likely that the small quantity of salts present was the limiting factor, it being perhaps insufficient to satisfy the necessary demands of the plant.

Calcium Nitrate and Magnesium Nitrate. Experiment 2

It was next desired to test the activity of root absorption in solutions of calcium and magnesium nitrates. Accordingly four groups of solutions were prepared having a total salt content of 120, 240, 360, and 480 N × 10^{-6}, respectively, each group consisting of the following numbers: 4/4 $Ca(NO_3)_2$, 2/4 $Ca(NO_3)_2$ + 2/4 $Mg(NO_3)_2$, 1/4 $Ca(NO_3)_2$ + 3/4 $Mg(NO_3)_2$, 1/10 $Ca(NO_3)_2$ + 9/10 $Mg(NO_3)_2$, and 4/4 $Mg(NO_3)_2$. As before, each solution received four seedlings and concentration changes were followed by means of a daily reading of the electrical conductivity. The daily concentration of each solution during the experimental period of 19 days is shown in figure 2.

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[6] Amer. Journ. Bot. 2: 1915.

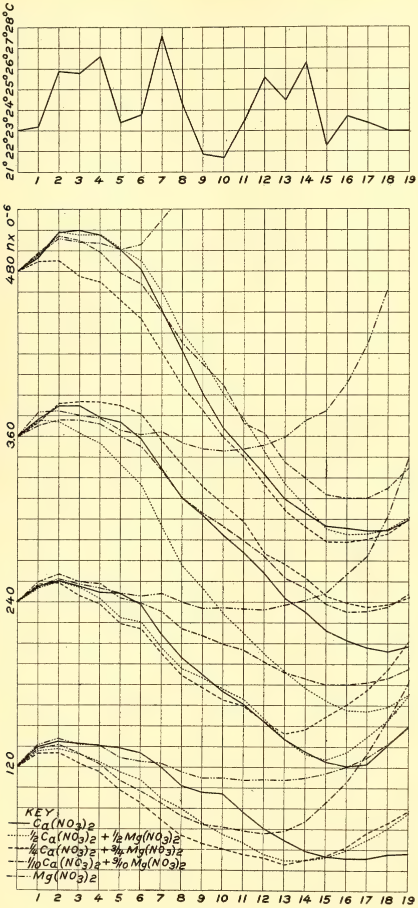

FIG. 2. Curves showing the change in concentration of the $Ca(NO_3)_2$ and $Mg(NO_3)_2$ solutions used in experiment 2.

In this series of cultures it was again noted that in general absorption increased with the increasing total salt concentration, although not proportionately. The $Mg(NO_3)_2$ solutions were an exception to this otherwise general rule, owing to the great toxicity of this salt.

In the 120 N × 10^{-6} group of solutions the solutions gained ions for a period of two or three days. It is probable that in both the Mg and the Ca solutions there was some excretion of salts from the plants, since nearly a week passed before this loss was made up, whereas the smaller apparent loss seen in the case of the mixtures might all be accounted for by the increase in the CO_2 content of the solutions. It will be noted that in the solution of $Ca(NO_3)_2$ after the initial period of about two days marked by an active loss of ions, absorption began to take place with increasing rapidity until near the end of the experiment a net decrease of nearly 90 N × 10^{-6} had taken place in the concentration of the solution. The record for $Mg(NO_3)_2$ was similar to that for $Ca(NO_3)_2$ for seven days after, which time absorption slackened and the solution stood at equilibrium with a small net absorption until near the end of the experimental period, when loss of salts from the roots set in and lasted until the experiment closed. In all mixed solutions a more active absorption was made during the first 12 days than in either of the unmixed solutions. It will be noted that the greatest net absorption took place from the solution containing 1/4 $Ca(NO_3)_2$ + 3/4 $Mg(NO_3)_2$ although late in the experiment the plants in that containing 2/4 $Ca(NO_3)_2$ + 2/4 $Mg(NO_3)_2$ reached practically the same maximum, exceeding that made in the 4/4 $Ca(NO_3)_2$ by a very small margin. The solution containing 1/10 $Ca(NO_3)_2$ + 9/10 $Mg(NO_3)_2$ showed a loss in concentration of over 60 N × 10^{-6} against about 12 N × 10^{-6} for 10/10 $Mg(NO_3)_2$. The net absorption from the 4/4 $Mg(NO_3)_2$ solution was multiplied five times by the replacement of one-tenth of the magnesium by calcium. In the 240 N × 10^{-6} group the characteristic features are: (1) the increased and nearly parallel absorption for thirteen days from the solutions containing 4/4 $Ca(NO_3)_2$, 2/4 $Ca(NO_3)_2$ + 2/4 $Mg(NO_3)_2$, and 1/4 $Ca(NO_3)_2$ + 3/4 $Mg(NO_3)_2$; (2) the lengthening of the period of active absorption with the increase of the quantity of $Ca(NO_3)_2$ present; (3) the failure of absorption in the solution containing 1/10 $Ca(NO_3)_2$ + 9/10 $Mg(NO_3)_2$ to keep its place relative to the other mixtures; and the long delayed and brief period of slight net absorption from the solution containing 4/4 $Mg(NO_3)_2$.

In the group having a total salt concentration of 360 N × 10^{-6} the introductory period during which the solutions gained ions was strongly marked in all solutions except that containing 2/4 $Ca(NO_3)_2$ + 2/4 $Mg(NO_3)_2$. In that solution net absorption became evident more quickly than in the other members of the group and the lead was held throughout the experiment. The net change in concentration of the solution containing 4/4 $Ca(NO_3)_2$ amounted to over 150 N × 10^{-6} a change exceeded only in the most favorable mixture, that containing 2/4 $Ca(NO_3)_2$ + 2/4 $Mg(NO_3)_2$. In this mixture the absorption corresponded to a change in concentration of about 200 N × 10^{-6} by the 17th day. The absorption from mixtures containing smaller proportions of $Ca(NO_3)_2$ was less than that from 4/4 $Ca(NO_3)_2$ solution. The $Mg(NO_3)_2$ solution showed the same features as heretofore.

In the group having a total salt concentration of 480 N × 10^{-6} it will be noted that in general the courses of all curves representing calcium-containing solutions agreed more closely than in the more dilute solutions, even that containing but 1/10 $Ca(NO_3)_2$ to 9/10 $Mg(NO_3)_2$ acting relatively more favorably than in the less concentrated solutions. Apparently the mixtures were approaching a situation in which the Ca demand was satisfied even in those containing the smaller proportions of this ion. In the 4/4 $Ca(NO_3)_2$ solution the maximum net absorption was attained as heretofore near the end of the experimental period. By the 18th day the concentration of the solution had diminished about 185 N × 10^{-6}. The most favorable nixture was that containing 1/4 $Ca(NO_3)_2$ + 3/4 $Mg(NO_3)_2$ from which the net absorption on the 15th day corresponded to a drop in concentration of about 200 N × 10^{-6}. It is clear that a toxic concentration of 4/4 $Mg(NO_3)_2$ had been reached since at the end of six days very marked leaching began, and at no time was there a net absorption.

Looking at this experiment as a whole a number of features attract attention. The contrast between $Ca(NO_3)_2$ and $Mg(NO_3)_2$ in regard to their influence on root absorption is very marked. The calcium salt favored this process in all concentrations here tested. Magnesium nitrate in no case supports more than a slight net absorption in the most favorable concentrations, and the poisonous action of the Mg ion promptly appeared at a concentration between 360 and 480 N × 10^{-6}. All mixtures with $Ca(NO_3)_2$ in all proportions were more freely absorbed than $Mg(NO_3)_2$ alone. The solutions most favorable for absorption contained 2/4 or 1/4 $Ca(NO_3)_2$. Although much less favorable for absorption than the solutions containing higher proportions of calcium nitrate, the solution containing but 1/10 $Ca(NO_3)_2$ to 9/10 $Mg(NO_3)_2$ was incomparably better than that containing 10/10 $Mg(NO_3)_2$. In its effect upon absorption, as upon root growth, a small amount of calcium has an effect all out of proportion to the quantity actually present.

Magnesium Nitrate and Potassium Nitrate. Experiment 3

In this experiment, as before, four groups of solutions were made up having a total salt content of 120, 240, 360, and 480 $N \times 10^{-6}$, respectively, each group containing solutions of the salts in the following proportions: 4/4 $Mg(NO_3)_2$, 3/4 $Mg(NO_3)_2$ + 1/4 KNO_3, 2/4 $Mg(NO_3)_2$ + 2/4 KNO_3, 1/4 $Mg(NO_3)_2$ + 3/4 KNO_3 and 4/4 KNO_3. As in experiment 1 the anion concentration was the same for the five solutions of each group. Since in the mixtures an ion with a valence of two had to be replaced by two ions with a valence of 1, the ratios of kations to anions formed the series 4 : 8, 5 : 8, 6 : 8, 7 : 8, and 8 : 8. The electrolytic conductivity of each solution was observed daily during an experimental period of 14 days.

In the series of curves shown in figure 3 a number of novel features appear. The very general tendency of the root absorption to increase with the increase of the total salt concentration, so clearly seen in the foregoing experiments, is here absent. The best absorption in 4/4 $Mg(NO_3)_2$ took place in the weak 120 $N \times 10^{-6}$ solution, although the 240 $N \times 10^{-6}$ solution was nearly as good. In the higher concentrations injury appeared only a few days after the experiment was begun as was indicated by a rapid loss of ions by the plants.

The best absorption in 4/4 KNO_3 was seen in the strongest solution where a distinct though brief net absorption phase lasted from the 6th to 8th days. A minimal net absorption was seen in the 320 $N \times 10^{-6}$ solution on the 7th day. In the weaker solutions there was no net absorption. The greatest loss of salts by the plants occurred in the weakest solution.

The roots made a net absorption in every mixture of whatever proportion or concentration. Among the mixtures with a total salt concentration of 120 $N \times 10^{-6}$ none showed as great absorption as the 4/4 $Mg(NO_3)_2$ solution. The roots showed a decreased absorption as the proportion of $Mg(NO_3)_2$ was reduced. In the mixtures having a total concentration of 240 N $\times 10^{-6}$ the best absorption took place in that containing 3/4 $Mg(NO_3)_2$ + KNO_3. Nearly as favorable was 1/4 $Mg(NO_3)_2$ + 3/4 KNO_3. These were both decidedly better than the pure $Mg(NO_3)_2$ solution. The greater loss of ions by roots in the mixture containing equal parts of the two salts is believed to be an aberrant result due perhaps to the use of less vigorous roots than those usually employed. Roots growing in mixtures having a total concentration of 360 N $\times 10^{-6}$ in every case absorbed more freely than from either of the unmixed solutions of the components. The 4/4 $Mg(NO_3)_2$ solution was clearly toxic while in 4/4 KNO_3 the plants even at the height of the absorption phase were hardly able to recover a quantity of electrolytes equal to that previously lost. In all the mixtures a marked net absorption took place although it was somewhat less than in the case of the 240 N $\times o^{-6}$ mixtures. The advantage seemed to lie with the solutions containing the lesser proportions of $Mg(NO_3)_2$.

In the group of solutions having a total salt content of 480 N $\times 10^{-6}$ the harmful action of 4/4 $Mg(NO_3)_2$ and of the mixture containing 3/4 $Mg(NO_3)_2$ was clearly marked on the 7th day. The higher concentration of the 4/4 KNO_3 solution seemed nevertheless to be more favorable to absorption than any of the lower concentrations, for a slight but undoubted net absorption of ions took place during the first

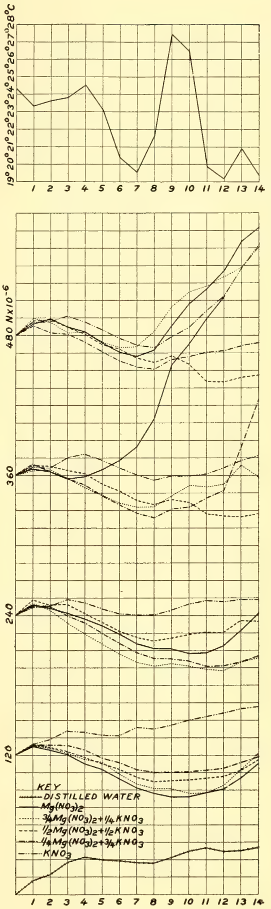

FIG. 3. Curves showing the change in concentration of the $Mg(NO_3)_2$ and KNO_3 solutions used in experiment 3.

week. After this period a rapid loss of ions by the plant set in. A marked and well sustained net absorption was seen in the mixtures containing 2/4 and 1/4 $Mg(NO_3)_2$, respectively, the maximum change in concentration being about 40 N × 10^{-6} on the 11th day in the solution containing equal parts of the two salts.

It is clear that of this pair of kations Mg^{++} is more favorable to root absorption in the concentrations here used than K^+. But for its relatively high degree of toxicity, which restricts life to a narrow range of concentrations, magnesium would bear a certain resemblance to calcium. Here as before potassium brings about a loss of ions by the plant. Nevertheless mixtures of the two nitrates are able to support a very considerable absorption, though it is little when compared with mixtures containing Ca ions.

The greatest absorption in the Mg–K series was made in the solution containing 3/4 $Mg(NO_3)_2$ + 1/4 KNO_3 in a total concentration of 240 N × 10^{-6}.

Mixtures of Mg and K ions showed in this experiment a marked superiority over either ion alone, as judged by the process of absorption.

SUMMARY OF RESULTS

The chief results obtained in this series of experiments with the white lupine may be summarized as follows:

1. When the nitrates of calcium, magnesium and potassium are offered in pairs in solutions varying in concentration from 120 N × 10^{-6} to 480 N × 10^{-6} it is usually the case that the roots absorb more electrolytes than from the pure solutions. It seems also true that for the range of concentration here concerned absorption tends to increase in mixtures as well as in pure solutions as the salt content increases. The case of magnesium salts offers an exception owing to the narrow range of physiologically useful concentrations of the Mg ion.

2. In mixtures of $Ca(NO_3)_2$ and KNO_3 the inimical effect of K ions on root absorption is seen in the high ratio of Ca to K required to give maximum absorption, viz., 3 Ca^{++} : 2 K^+ (3 $Ca(NO_3)_2$ + 1/4 KNO_3) in all concentrations tested. The value of a small amount of K ions is however proved by the excess of absorption in the mixture over that in $Ca(NO_3)_2$ alone. The absolute amount of Ca present in mixtures seems to be of great influence since as the proportion of Ca increases in the greater dilutions, absorption is increased. The favorable influence of Ca ions is therefore striking in the mixtures as well as in the pure solutions.

3. In mixtures of $Ca(NO_3)_2$ and $Mg(NO_3)_2$ the greater absorption of the Mg ion in comparison with the K ion appears in the greater proportion of Mg to Ca seen in the most favorable ratio. The greatest absorption is found in the ratios 2 Ca^{++} : 2 Mg^{++} or 1 Ca^{++} : 3 Mg^{++} in the concentrations here tested. The great significance of even a small proportion of Ca is seen in the relatively high absorption made in a mixture containing 1 Ca to 9 Mg at the highest concentrations here tested.

4. In pure solutions the Mg ion is much more favorable to absorption than the K ion in the weaker concentrations while the K ion is more favorable in the highest concentration. They are absorbed to about the same extent at a concentration of $360 N \times 10^{-6}$. Absorption from mixtures exceeds that from either pure solution except in the weakest concentrations. Although a most favorable ratio can hardly be designated, the general tendency of the results seems to indicate that a high proportion of Mg is more favorable in weaker concentrations.

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Bureau of Plant Industry.

THE PROBABLE NON-VALIDITY OF THE GENERA BOTRYODIPLODIA, DIPLODIELLA, CHAETODIPLODIA, AND LASIODIPLODIA

J. J. Taubenhaus

In the course of my investigations on the diseases of the sweet potato (25) the name Java black rot was applied to a disease caused by the fungus *Lasiodiplodia tubericola* E. & E. The investigation plainly showed that the above fungus behaved very much like a Diplodia. Experiments were undertaken to prove the pathogenicity of the fungus, its relationship to the genera Diplodia, Chaetodiplodia, Botryodiplodia, and Diplodiella.

The genus Diplodia founded by Fries (9) in 1849 shows the following characteristics: Pycnidia scattered, subcutaneous to erumpent, black, characteristically papillate at the mouth, spores one-septate, brown to dark. The genus Botryodiplodia founded by Saccardo (20) in 1880 is described as follows: Pycnidia caespitose (clustered) erumpent, and in a stroma; hairy or hairless, spores one-septate, dark. The genus Diplodiella founded by Karsten (14) in 1884 resembles Diplodia in every way except that the pycnidia are superficial instead of erumpent. The genus Chaetodiplodia was also founded by Karsten (15) in the same year; it resembles Diplodia in that the pycnidia are scattered. In this genus however they possess bristles, or hair; spores one-septate, dark. The genus Lasiodiplodia was created by Ellis and Everhart (6) in 1896 and described as follows: Pycnidia and subicle are enclosed in a hemispherical stroma. In addition there are paraphyses intermingled with the basidia of the sporules in the pycnidia; otherwise as in Diplodia.

From the above descriptions it is evident that the classification is without proper basis. To separate Diplodiella from Diplodia because the pycnidia are erumpent in the latter, while they are superficial in the former is not justified from a generic standpoint. The same is true for the other genera here mentioned. From what follows, it is evident that with the exception of the genus Diplodia, which was first described, the others are not valid.

Historical.—While working on a branch and trunk disease of cocoa, Jonge and Dorst (13) in 1909, carefully studied the causative fungus *Diplodia cacaoicola* Henn. They noticed that although the organism seemingly belonged to the genus Diplodia, under certain cultural conditions its pycnidia would be hairy and possess paraphyses intermingled with the basidia of the sporules. Different workers have actually placed this fungus under the different genera here mentioned, as will be seen later under its synonymy. Jonge and Dorst (13) further found that if the diseased specimens of cocoa were placed in moist surroundings, hair developed on the pycnidia, whereas dryness tended to suppress them. From these studies they concluded that the genera Chaetodiplodia and Lasiodiplodia are not valid.

In the same year (1909) Griffon and Maublanc (10), while working on a similar cocoa disease, came to conclusions similar to those of Jonge and Dorst (13). Griffon and Maublanc placed the fungus *Diplodia cacaoicola* Henn. in the genus Lasiodiplodia and named it *L. theobromae* (Patt.) Griff. & Maubl. Bancroft (2) (1911) in his work on a para rubber and cocoa disease, found that the causative fungus, *Diplodia cacaoicola* Henn. was so variable that it could easily be placed in any one of the genera Botryodiplodia, Macrophoma, and Lasiodiplodia. Bancroft, however, found the ascigerous stage of the fungus, which he named *Thyridaria tarda* Banc. That the presence or absence of paraphyses in pycnidia or perithecia have no taxonomic value has also been indicated by Shear and Wood (22). "Their presence or absence (paraphyses) does not seem to be sufficiently constant to be of much taxonomic value."

Present work.—As previously stated these investigations are an outgrowth of studies on *Lasiodiplodia tubericola* E.&E., the cause of a sweet potato disease. In order to determine the relationship of the genus Lasiodiplodia to the genera Diplodia, Diplodiella, Chaetodiplodia, and Botryodiplodia, the following experiments were carried out. A large number of apparently healthy sweet potato roots were divided into five lots. These were carefully washed in tap water, then disinfected by being plunged in a 5 percent formaldehyde solution for ten minutes. Each lot was then carefully dried with clean cheese cloth, placed in flat moist chambers, and kept one week in the laboratory. Any root which showed signs of decay because of internal infection was discarded; infection in such cases was chiefly soft rot, *Rhizopus nigricans* Ehr. At the end of the week ten roots were finally placed in each lot. Lot I was used as a check. Lot II was inoculated with a pure culture of *Lasiodiplodia tubericola* E. & E. The method of the inoculations here recorded was to insert bits of mycelium of pure culture into slits in the epidermis and cambium made with a flamed and cooled scalpel. Lot III was inoculated with a pure culture of *Diplodia gossypii* Zim. obtained from Dr. Edgerton; Lot IV was inoculated with a pure culture of *Diplodia natalensis* Pole Evans; and Lot V was inoculated with a pure culture of *Lasiodiplodia theobromae* (Patt.) Griff. and Maubl. (*Lasiodiplodia nigra* Appel & Laub.). Attempts to secure cultures of Diplodiella, Chaetodiplodia and Botryodiplodia failed. The results of the above inoculations were all positive. Inoculations with *Lasiodiplodia tubericola* on the sweet potato reproduced the typical Java black rot (figs. 7 and 8) within ten to twenty days. The period of incubation was from eight to nine days. The same was true for all the other fungi tried out. The gross symptoms produced differed so little from the typical Java black rot that it was impossible to tell them apart (figs. 1 and 2). Specimens of sweet potatoes inoculated with *Diplodia gossypii* and *Lasiodiplodia theobromae* were submitted to some of our leading mycologists without having been told of these inoculations, and they pronounced them similar to *Lasiodiplodia tubericola.* Sweet potatoes inoculated with *Diplodia natalensis* produced symptoms of Java black rot, but the fungus failed to form fertile pycnidia. While the gross symptoms were practically alike, a closer examination showed some differences. When the fungus *Lasiodiplodia tubericola* is inoculated into the sweet potato, the parasite emits from its pycnidia long strings of hyalin, one-celled Macrophoma spores, followed by the emission of black powdery heaps of dark one-septate spores of the Diplodia type (fig. 4). The fungus *Diplodia gossypii* on sweet potatoes did not emit the whitish strings of Macrophoma spores, but only produced black powdery heaps of spores of the Diplodia type. The same was true when *Lasiodiplodia theobromae* was used.

In shape, color, and measurements the pycnospores from *Lasiodiplodia tubericola* on sweet potato could hardly be distinguished from the pycnospores of *Diplodia gossypii*, and *L. theobromae* on the same host. It is evident that the hyalin one-celled Macrophoma spores of *Lasiodiplodia tubericola* are immature Diplodia spores. These turn brown with age and become one-septate, although they are capable of germination while young.

Dilution cultures carefully made of the Macrophoma spores produce a growth typical of *Lasiodiplodia tubericola*, and when inoculated on the sweet potato produces the typical Java black rot. Similar conclusions have been reached by Emerson (7) and several others. According to Jonge and Dorst (13), *Lasiodiplodia theobromae* on cocoa emits from its pycnidia white, strongly curled tendrils made up of hyaline one-celled spores of the Macrophoma type. As alreadys een, this same fungus on the sweet potato produces only the Diplodia type of spores. This clearly indicates the influence of the host as a factor in promoting or suppressing the Macrophoma stage.

In order to study further the relationship of the genera here under discussion, sweet potato material infected with the above fungi was fixed in a chrom-acetic solution of medium strength, then sectioned and stained with safranin and gentian violet. It is only through a pathological study of the host infected with these fungi that the relationship of the supposed established genera is brought to light. As already stated, when sweet potatoes are inoculated with the fungus *Lasiodiplodia tubericola*, pycnidia are produced with or without paraphyses (figs. 14 and 15). The same also holds true for *Diplodia gossypii* on the sweet potato, *i. e.*, there are many pycnidia with and without paraphyses.

A study of the sectioned material further reveals the fact that some of the pycnidia of *Lasiodiplodia tubericola* on the sweet potato are either borne singly or in groups (fig. 15), and seem to be embedded in a stroma (figs. 14 and 19). This also holds true for *Diplodia gossypii* on sweet potato. It will be remembered that Diplodia and Lasiodiplodia differ in that the latter possess paraphyses and the pycnidia are embedded in groups in a stroma; Diplodia has no paraphyses and the pycnidia are borne singly. This at once shows how artificial is the separation of these two genera. *Diplodia gossypii* in the sweet potato produces paraphyses (fig. 21) and the pycnidia are either borne singly or in groups (figs. 22 and 26), as is the case with *Lasiodiplodia tubericola*. Because of these facts we are justified in dispensing with the genus Lasiodiplodia. Jonge and Dorst (13) reached a similar conclusion in their work on the fungus *Lasiodiplodia theobromae* as did also Griffon and Maublanc (10).

It has already been stated that Chaetodiplodia is distinguished from Diplodia in that the neck of the pycnidium is hirsute, whereas in the latter it is without hair, a distinction which is very artificial.

Further microscopical study of our sectioned sweet potato material shows that the necks of nearly all the pycnidia of *Lasiodiplodia theobromae* have hairs (fig. 23). The same is true for *Lasiodiplodia tubericola* (figs. 18) and *Diplodia natalensis* (fig. 25). In the latter two, however, the hairiness is not so pronounced and sometimes it may be absent altogether. Similar observations were made by Griffon and Maublanc (10) and by others. From this it is evident that the genus Chaetodiplodia cannot stand and that Diplodia alone should be retained.

According to Jonge and Dorst (13) and others, the hairiness of the neck of the pycnidia is largely dependent upon cultural conditions. They state that lack of moisture tends to produce glabrous necks and much moisture has the opposite effect. My studies do not support these conclusions, since the inoculated sweet potatoes were kept in moist chambers. Under similar conditions *Lasiodiplodia theobromae* produced hirsute pycnidia and *L. tubericola* produced either glabrous or sparingly hirsute pycnidia. The determining factor in the production of hirsute or glabrous pycnidia is yet to be worked out.

Botryodiplodia was distinguished from Lasiodiplodia in the arrangement of the pycnidia. In the former they are cæspitose, in the latter they are borne in a stroma. It has been shown that the genus Lasiodiplodia is untenable. In further study of the sectioned material it is seen that the pycnidia in *L. tubericola* may be borne singly, in a stroma, or cespitose (fig. 14). The same also holds true for *Diplodia gossypii* (fig. 22). This therefore clearly indicates that the genus Botryodiplodia, too, should be dropped, its characteristics being included in the genus Diplodia.

The genus Diplodiella differs supposedly from the other genera here mentioned in that the pycnidia are superficial. This distinction does not hold. In further studying sectioned material, it is seen that *Diplodia gossypii* often produces a number of pycnidia which are distinctly superficial (fig. 22). Because of these facts the genus Diplodiella is not tenable. It is evident that the genus Diplodia under different hosts and climate may take on some or all of the characteristics of the more recent genera Chaetodiplodia, Lasiodiplodia, Botryodiplodia, and Diplodiella. The common fungus *Lasiodiplodia theobromae* has actually been placed by different workers in all of these genera except Diplodiella.

Griffon and Maublanc (10) suggest that *Diplodia tubericola* (E. &

E.) Taub. may possibly be the same as *L. theobromae*. From the above studies, *Diplodia tubericola* is seen to differ from *L. theobromae* in the following manner: *Diplodia tubericola* as already stated, produces pycnidia throughout all parts of the infected sweet potato (fig. 16). Moreover, the pycnidia while hirsute are often sparingly so. In *L. theobromae* there are few or no pycnidia in the interior of the host, and the pycnidial necks are strongly hirsute (fig. 23). *Diplodia gossypii* is also distinct from the above two species, as will be seen by comparing figures 20, 21, 22 with figures 14, 17, and 19. Supported by the above studies the genus Diplodia includes the characteristics upon which these other genera, viz.; Chaetodiplodia, Lasiodiplodia, Botryodiplodia, and Diplodiella are based, hence, following the rule of priority, they are not tenable, and all the species in these genera become species of Diplodia. Further work will probably show that the genera Rhynchodiplodia and Pellionellia may likewise be referred to the genus Diplodia. The following is a somewhat broadened description of the genus Diplodia:

Diplodia Fries.—Pycnidia black, subcutaneous to erumpent or superficial, scattered or in groups, cæspitose or in a stroma; hirsute or glabrous, paraphyses present or absent, spores hyaline, one-celled when young but one-septate, brown to dark when mature.

SUMMARY

Inoculations with two species of Lasiodiplodia and two species of Diplodia have brought out the following facts: The genus Diplodia is very variable. The fungus *Diplodia gossypii* for instance, when inoculated on the sweet potato, will show all the characteristics of the supposed genera Lasiodiplodia, Chaetodiplodia, Botryodiplodia, and Diplodiella. The same is also true when the Lasiodiplodias are inoculated on the sweet potato. From this it is therefore concluded that because of its priority the genus Diplodia alone should be retained, while the genera Lasiodiplodia, Chaetodiplodia, Botryodiplodia, and Diplodiella are not tenable and their species should be placed in the genus Diplodia. It is very probable that further work will show the necessity of abolishing the genera Rhyncodiplodia and Pellioniella. It seems probable also that more work will further reduce the large number of species of Diplodia.

Slides of sectioned and stained material illustrating the above studies will be deposited at the Delaware Agricultural Experiment

Station; the Department of Botany, University of Pennsylvania; and the National Museum at Washington, D. C.

Delaware College Agricultural Experiment Station,
Newark, Delaware.

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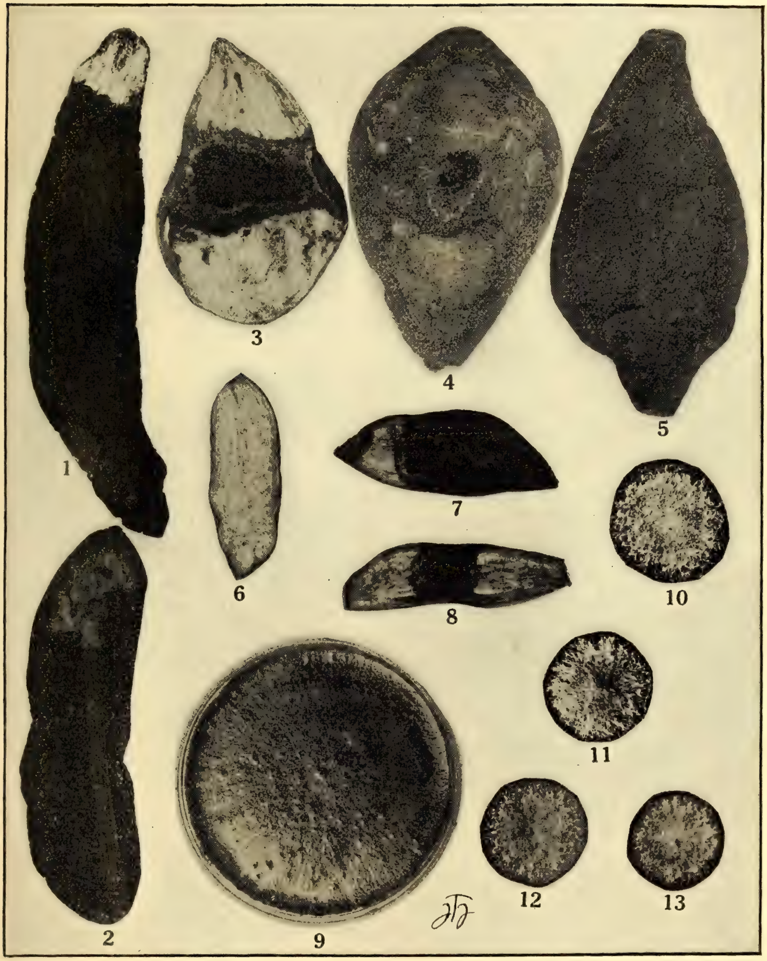
[1] References marked with * were not seen by the author.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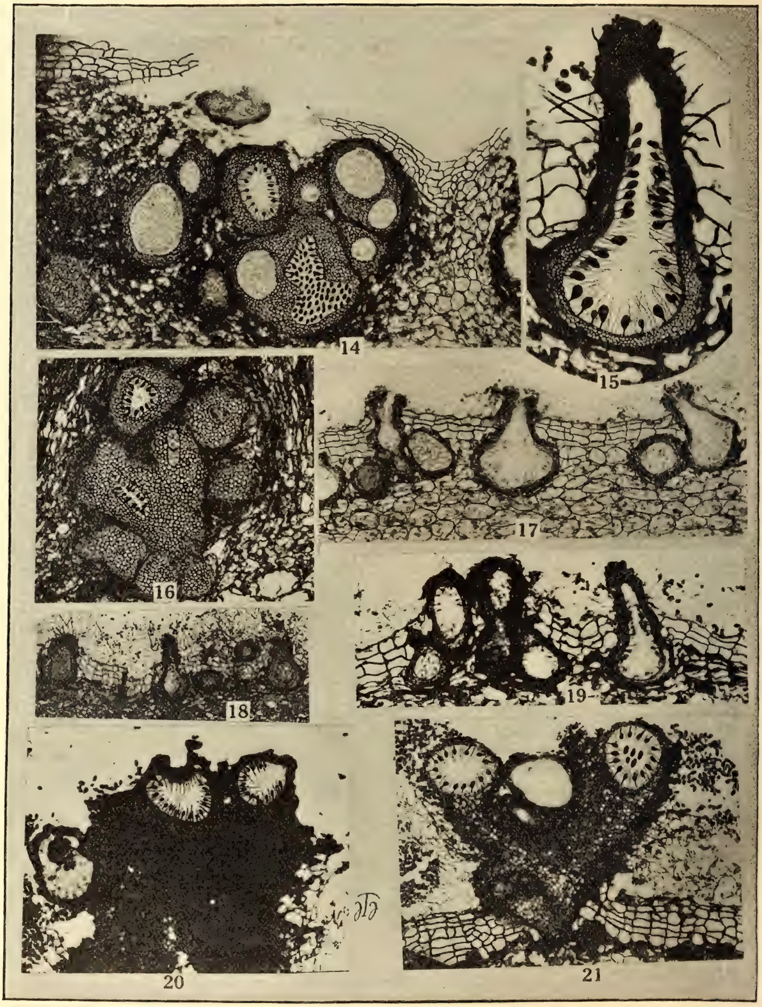
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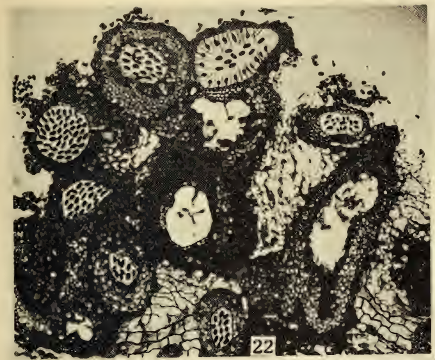
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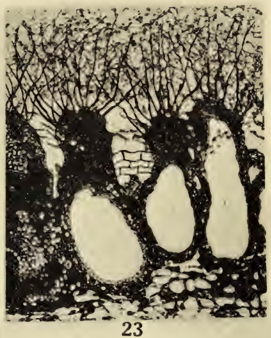
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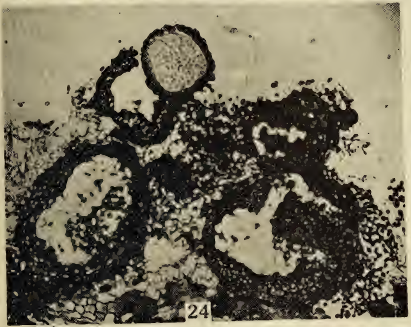
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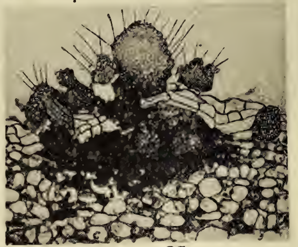
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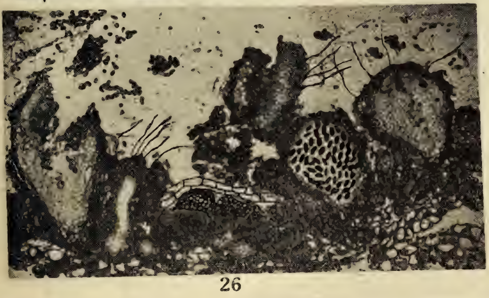
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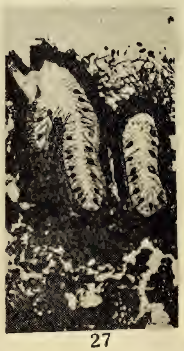
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TAUBENHAUS: DIPLODIA.

26. **Taubenhaus, J. J.** The Non-validity of the Genus Lasiodiplodia (abs.). Phytopathology 4: 47. 1914.

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EXPLANATION OF PLATES XII–XIV

FIG. 1. Sweet potato inoculated with Diplodia gossypii.

FIG. 2. Sweet potato inoculated with Lasiodiplodia theobromae.

FIG. 3. Cross section of sweet potato inoculated with D. tubericola.

FIG. 4. Surface view of same potato as in fig. 3.

FIG. 6. Check, healthy sweet potato.

FIGS. 7 AND 8. Sweet potatoes inoculated with L. tubericola.

FIG. 9. Pure culture of D. tubericola, showing formation of pycnidia in plate.

FIG. 10. Young culture of D. tubericola 3 days old.

FIG. 11. Young culture of D. theobromae, same age as in fig. 10.

FIG. 12. Young culture of D. natalensis, same age.

FIG. 13. Young culture of D. gossypii, same age.

FIG. 14.[2] Photomicrograph of a sweet potato section inoculated with D. tubericola showing a group of pycnidia,—with and without paraphyses.

FIG. 15. Photomicrograph of a sweet potato section inoculated with D. tubericola showing a single pycnidium with paraphyses and hair at its neck.

FIG. 16. Same material as fig. 14, showing pycnidia within the host.

FIG. 17. Same material as fig. 14, showing scattered pycnidia without hair.

FIG. 19. Same material as fig. 14, showing pycnidia single and in groups.

FIG. 18. Same material as fig. 14, showing hairy necked pycnidia.

FIGS. 20 AND 21. · Photomicrograph of a section of sweet potato inoculated with Diplodia gossypii, showing pycnidia embedded in a stroma, with paraphyses in the pycnidia.

FIGS. 22 AND 24. Photomicrograph of a section of sweet potato inoculated with Diplodia gossypii, showing cespitose pycnidia borne upon the epidermis of the host as is the case with Diplodiella. In fig. 22, paraphyses are also seen in one pycnidium.

FIG. 26. Same material as fig. 22, showing sparingly hirsute pycnidia.

FIG. 27. Same material as fig. 22, showing paraphyses in the pycnidia.

FIG. 23. Photomicrograph of a section of sweet potato inoculated with L. theobromae, showing hirsute pycnidia.

FIG. 25. Photomicrograph of a section of sweet potato inoculated with Diplodia natalensis, showing hirsute sterile pycnidia.

FIGS. 14, 15, 16, 20, 21, 22, 23, 25, 26 and 27 were retouched with pen and ink in order better to show paraphyses, or hair, which the camera has failed to reproduce.

[2] Thanks are due to Dr. T. F. Manns for help rendered in making figs. 14 to 27.

FACTORS INFLUENCING FLOWER SIZE IN NICOTIANA WITH SPECIAL REFERENCE TO QUESTIONS OF INHERITANCE[1]

T. H. Goodspeed and R. E. Clausen

I. Introduction

During the past five years the inheritance of flower size has been of interest in connection with the study of hybrids made in the University of California Botanical Garden between various pure lines of Nicotiana species and varieties. Three flower size varieties of *N. acuminata* (Graham) Hook. (Setchell, 1912, p. 23) were crossed back and forth and the hybrids examined up to and including F_3 (cf. Goodspeed, 1912 and 1913, (1)); species hybrids between *N. sylvestris* Speg. & Comes (Setchell, loc. cit., p. 29) and a number of *N. Tabacum* L. varieties (ibid., pp. 3–11) have also been under observation and their F_1 flower size and the size of the flowers in the case of crosses of the parents back onto the hybrids have been studied in detail. At the present time a study is being made of the flower size of a self-fertile hybrid between *N. Langsdorfii* Weinm. and *N. Langsdorfii* var. *grandiflora* Comes (Setchell, 1912, p. 15) which should be of interest because of the marked difference in flower size of the parental types.

In the collection of some 25,000 measurements on the length of corolla tube and the spread of corolla limb of flowers on the various hybrids above noted certain very definite difficulties have been encountered. These difficulties have had their influence as well in determining the technique of taking the measurements as in directly influencing the value and interpretation of the general results obtained. East (1913, pp. 177 and 178) finds reason to believe that corolla size, which he recognizes as a "character complex," is "comparatively constant under all conditions attending development." In this connection he refers to the well-known fact that other size characters, in contrast to this comparative stability of corolla size, are subject to a

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[1] The experiments herein reported upon were assisted by that portion of the grants to the University of California, College of Agriculture, under the Adams Fund, which was put at the disposal of Prof. W. A. Setchell.

rather greater degree of fluctuating variability under similar conditions attending development. Thus we read that, "During the past four years I have grown about 20 species of Nicotiana in considerable numbers. They have been grown under very diverse conditions. Some have been starved in four-inch pots, others have had the best of greenhouse treatment; some have had poor field conditions, others have had all field conditions practically at their best. The height of the plants, the size of the leaves, and similar size complexes have varied enormously, but the size of the corollas has varied scarcely at all. For example, plants of *N. sylvestris* Speg. & Comes grown to maturity in four-inch pots produced no leaves longer than 7 inches. On the other hand sister plants of the same pure line produced leaves 30 inches long in the field. Both series, however, produced flowers with the same length and spread of corolla. Furthermore, cuttings from 20 of the field plants reported in this study were rooted and grown in small pots in the greenhouse. Their blossoms were the same size as those of the field plants from which they came." The data herein to be listed seem, in our opinion, to demonstrate that in the Nicotiana cultures grown in the University of California Botanical Garden a rather different condition of affairs prevails so far as the "character complex," corolla size, is concerned. East also finds (ibid., p. 181) that in general "corolla spread is . . . correlated with corolla length." As a broad generalization this statement is undoubtedly correct. On the other hand, the fact that in our experience corolla length and corolla spread behave in a different manner with reference to their stability under "the conditions attending development," suggests that the assumption of a too definite correlation when attempting to interpret the results of studies of flower size inheritance in Nicotiana must be very guardedly indulged in. The fact that in our experience corolla size does vary distinctly under some of the most normal and inherent conditions attending development and that corolla spread and corolla length do not respond alike in this respect has in a great measure been the cause of the difficulties in technique and interpretation above mentioned.

The data presented in this report were collected in connection with a number of separate and distinct experiments, and pertinent data upon an almost equal number of plants were also available. It is the purpose of the present report to illustrate by the presentation of the results, actually accumulated in the field, the effects of various environmental and especially developmental factors in determining the expression of the "corolla complex" in Nicotiana. The extent of the data and the nature of the conclusions derived therefrom make it seem possible to establish tentative criteria in keeping with which flower size investigations, in Nicotiana at least, should be carried on and interpreted. In the present report no effort will be made to illustrate or to discuss specifically the nature or manner of the inheritance of flower size in Nicotiana. Further reports in preparation aim to take up this question in detail and to discuss it on the basis of the various considerations herein to be emphasized.

II. Experimental Material

The data presented herewith have to do with measurements on the size of flowers produced by:

1. *N. Tabacum* var. *macrophylla* Comes (Setchell, loc. cit. p. 8) in the growing season 1913. This *N. Tabacum* variety has been grown here for 6 years in the pure line and will be referred to under its University of California Botanical Garden number, U. C. B. G. 22/07.

2. The F_1 hybrid *N. Tabacum* var. *macrophylla* (22/07) ♀ × *N. sylvestris* (ibid., p. 29) ♂ which will be referred to as F_1 H_{38}. This cross was made by Professor W. A. Setchell in 1911 and the flowers on 25 F_1 hybrid plants were first measured in 1912. These F_1 plants were cut back early in 1913 and came into full flower during May of the same year. Their flowers were measured in large numbers. A second sowing of the seed of the original cross was made in 1913 and the flowers on 10 plants were measured that season. We have thus data upon the flower size of: 12 F_1 H_{38}, plants from the first sowing of the hybrid seed; 13 F_1 H_{38}, plants of 12 F_1 H_{38} coming up on their own roots; 13 (11) F_1 H_{38}, plants from a second sowing of the original hybrid seed.

Thus plant No. 1 of 13 F_1 H_{38} is plant No. 1 of 12 F_1 H_{38} growing the second year on its own roots, while plant No. 1 of 13 (11) F_1 H_{38} cannot be compared directly with any plant of 12 F_1 H_{38} or 13 F_1 H_{38}, although it is genetically of exactly the same constitution.

3. F_1 H_{137}, which is the reciprocal of F_1 H_{38}. This cross was made in 1912 and flowers on F_1 plants were measured in 1913.

4. The F_1 hybrid produced by crossing F_1 of the hybrid *N. Tabacum* "Maryland" (ibid., p. 5) ♀ × *N. Tabacum* "Cavala" ♂ with *N. sylvestris*. The garden number of this cross is H_{44}. It was made in

1911 by Professor Setchell and F_1 flowers were measured in 1912. These plants, also, were cut back early in 1913 and were fully in flower during May of the same year. Cuttings of certain of the F_1 H_{44} plants were rooted in the greenhouse during the fall and flowered under glass in the early spring. Another sowing of H_{44} was made in 1913 but no measurements were taken upon the flowers of these plants.

The technique of taking the measurements on spread or diameter of corolla limb was the same as that used in the previous studies on the inheritance of flower size in Nicotiana (cf. Goodspeed, 1912, p. 138). The measurements of spread of corolla limb herein listed were taken without the use of any holder and with the corolla limb supported between the thumb and forefinger. Length of corolla tube was taken to be the distance from the point of union of calyx and pedicel to the point where the under side of the reflexed limb joins the corolla tube. In the case of species of Nicotiana here dealt with the flowers were fully open throughout the day as contrasted with the more or less vespertine *N. acuminata* varieties and their hybrids, which were studied previously.

III. Factors Influencing Flower Size

1. "*Age of Plant*" *Factor*

The heading "'Age of Plant' Factor" has to do with the fact that a tobacco plant during the first few days after coming into flower bears flowers which differ in size from the flowers which it bears later in the growing season normal for this particular species or variety. It also serves as a heading for the discussion of the fact that the flowers borne on plants cut back and growing on their own roots a second season differ in size from the flowers borne on the same plants the first year. The following tables (Tables I to V) are intended to illustrate the situation with reference to early and late flowering on the same plant. These are condensed statements of the full records and have to do with the frequency distributions for the entire population in each case. On account of their extent it would hardly be possible to include the complete records for each plant on the different dates on which the flowers were measured.

In the tables the plants are arranged according to the mean flower size of the particular date or period in question. The mean flower size in every case for each date was calculated and also a mean for the period during which the measurements were taken. This last is the mean designated under "means for totals" in the tables. It was calculated simply by taking the average flower size of all the flowers measured during the entire period. On account of the different numbers of flowers measured on the different dates, this calculation would differ somewhat from a mean based on equal weighting of each date, but such a procedure does not appear to have any particular advantage for the purposes of this discussion.

TABLE I*a*

Frequency Distributions for Spread of Corolla in U. C. B. G. $13^{22}/_{07}$,

Designation	Class Centers in Millimeters														No.	Mean	Coef. Var.
	19	20	21	22	23	24	25	26	27	28	29	30	31	32			
Means for totals..........							7	25	28	7	6	1	1		75	26.83±.09	4.45±.24
Means for first dates......							1		13	25	25	8	1	2	75	28.48±.09	4.11±.23
Means for last dates.......	1	3	8	11	15	5	10	10	1	3	3	3	2		75	24.19±.22	11.59±.64
Means for different dates ..		1	2	4	6	2	6	6	12	18	12	4		2	75	26.69±.20	9.77±.54

Tables I*a* and I*b* give the frequency distributions for spread and length of corolla, respectively, of 13 22/07 under designations which are in part at least self-explanatory. For example the line in Table I*a* designated "means for totals" gives the distribution of the means of the 75 plants under consideration for the entire number of measurements taken. Thus for Plant No. 1 of 13 22/07, 28.73 mm. represents the mean size of corolla spread as determined from the measurements of flowers on seven different dates from July 28 to September 22, and including a few flowers late in the season from the terminal inflorescence. In a few cases some open flowers not yet shedding pollen have been included in these calculations. An average of about 40 flowers per plant was measured during this period, not more than 80 nor less than 25 on any one plant. The "means for first dates" simply gives the distribution of means calculated from the flowers measured on the first date and likewise the "means for last dates" gives the distribution of means calculated from the flower measurements on the last date. The distribution designated "means for different dates" is one such as might be obtained if a large number of plants were measured, each on a single date; *i. e.*, if a worker began measuring the flowers on his plants at a certain date and gradually worked through the lot during a period corresponding to the one considered here. This distribution was obtained in this particular instance by grouping the means for the first 25 plants on the first dates, the second 25 on a mid-date, and the third 25 on the last date together in one distribution. Table I*b* has to do with corolla tube length of the same plants, and the same explanations of course apply to it.

TABLE I*b*

Frequency Distributions for Length of Corollas in U. C. B. G. $13^{22}/_{07}$,

Designation	Class Centers in Millimeters											No.	Mean	Coef. Var.
	38	39	40	41	42	43	44	45	46	47	48			
Means for totals.....				1	3	6	34	28	2	1		75	44.27±.07	2.13±.12
Means for first dates .						1	5	29	29	10	1	75	45.60±.07	1.96±.11
Means for last dates .	2	2	10	17	9	15	8	10	1	1		75	42.25±.15	4.58±.25
Means for different dates............	2	1	5	6	4	7	8	24	13	3	2	75	43.96±.18	5.18±.29

The period during which measurements were taken was from July 28 to August 30, although a few of the plants were measured again on September 22. Measurements of flowers borne among the developing seed capsules on the terminal inflorescence and also of open flowers which had not yet begun to shed pollen, when such were taken, were separately designated in the records. These classes of flowers show significant differences in flower size, as will be shown later on in this paper, and a haphazard selection of the flowers to be measured might easily give undue prominence to some one of these classes. They were not however excluded in the calculation of the total mean for each plant, but the error introduced in this manner is very slight on account of the small number measured in proportion to those fully open and borne on the later flowering laterals.

Certain difficulties and in some cases the arbitrary nature of the groupings are quite evident. As has been noted the measurements were taken in connection with a number of separate experiments and obviously the dates chosen for making the measurements were dictated by convenience rather than with the present use of the data in mind. The reason for the groupings will be explained in the pages that follow and the fact that, notwithstanding the above situation, the data bring out the facts to which we desire to call attention is sufficient justification for any slight discrepancies which may be apparent.

Table I*a* brings out the fact that the mean spread of corolla limb in the case of the means for 75 plants of *N. Tabacum* var. *macrophylla* (U. C. B. G. 22/07) is 26.83 ± .09 mm., and the range of fluctuation is 7 mm., from 25 to 31 mm. On the other hand, the total range in corolla spread as shown by the complete records amounted to 18 mm., from 18 to 35 mm.; and for individual plants as high as 14 mm. Similarly for length of corolla tube, as shown in Table I*b*, the mean was 44.27 ± .07 mm. with a range of fluctuation of 7 mm., from 41 to 47 mm. The complete records, again, show a total range of 18 mm., from 33 to 50 mm., for length of corolla tube. These facts are based upon measurements continued for nearly one month or in a few cases for over one month of the blooming period normal for this species and variety. If now the mean flower size is determined on the basis of measurements taken during the first 3 or 4 days of this period, the mean spread of corolla is 28.48 ± .09 mm., as contrasted with 26.83 ± .09 mm., and the length of corolla is 45.60 ± .07 mm. as contrasted with 44.27 ± .07 mm. Similarly if the mean is determined by measurements taken on all the plants at the end of this period, the mean corolla spread is 24.19 ± .22 mm. as compared with the mean for totals of 26.83 ± .09 mm. and the mean for the first dates of 28.48 ± .09 mm. In the same way for the length of corolla we have a mean of 42.25 ± .15 mm. at the end of the season, while the mean for totals is 44.27 ± .07 mm., and the mean at the beginning of the season is 45.60 ± .07 mm. If in the third place the mean is derived by measuring flowers on the first third of the plants early in the season, on the second third in the middle of the flowering season, and on the remainder of the plants toward the end of the season, we have for spread a mean of 26.69 ± .20 mm. which, as would be expected, is practically the same as the mean for totals 26.83 ± .09 mm., but differs from the means for the first and last dates which are 28.48 ± .09 mm. and 24.19 ± .22 mm., respectively. Similarly with reference to length of corolla we have a mean of 43.96 ± .18 mm. for such a distribution which is very close to the mean for totals of 44.27 ± .07 mm., but is distinct from the means of the first or last dates which are 45.60 ± .07 mm. and 42.25 ± .15 mm., respectively. The amount of these differences and their probable errors are tabulated in Table 6, where they may be compared with similar differences shown in other series of plants.

The differences between the means according to different times of measurement and different modes of arrangement furnish the basis for the more significant differences in variability under these same conditions. Thus we see that although the mean for "different dates," 26.69 ± .20 mm., does not differ sensibly from the "mean for totals" of 26.83 ± .09 mm., yet the coefficient of variability for the former distribution is over twice as great as in the latter case. The difference between the two is 5.32 ± .59 per cent, which is certainly large enough in comparison with its probable error to acquire considerable significance. The proportionate increase in variability for the corresponding length arrays is very slightly higher than that for width arrays, but for all practical purposes the same. Here again the difference between the two coefficients of variability, 3.05 ± .28 per cent is large enough to be of unquestionable significance. In figures 1 and 2 are given a graphic representation of the facts brought out in these tables.

It seems fair to assume that to determine the constants for flower size of a large series of plants, an F_2 population for example, the flowers on the plants would naturally be measured either all of them during the beginning of the flowering season, all toward the end of the season, or most probably in some such fashion as is expressed in the distribution of "different dates." Peculiarly enough published reports give us no account of the way in which the measurements of flower size were collected. It seems fair, however, to assume that in field work measurements of 25 flowers should represent the minimum required to give even an approximately fair mean flower size for a given plant. On this basis to any one familiar with the taking of such measurements the number of operations necessary to obtain the mean distribution of flower size in a representative F_2 population represents a stupendous amount of time. With anything like the normal amount of assistance available it hardly seems possible that any such number of measurements could be accumulated in less than a month and a half; and, if so, some of the plants would be measured at the beginning, others at the middle, and still others at the end of the period of flowering. Such a system would merely give a representative mean and would not give a body of data suitable for the calculation of other constants. The only other alternative involves the measuring of a few flowers on each plant during the first week of the season and repeating this process each week throughout the season. Even this, however, would involve the necessity of taking a larger number of measurements than is ordinarily possible during such a time. We feel that a distribution based upon means derived from measurements taken throughout the growing and flowering season is, with one possible exception to be noted in the following pages, the only representative distribution. Such a distribution expresses the entire demonstrable series of developmental changes in the flower which accompanies the normal ontogenetic plant processes. It is hardly necessary to point out the bearing which results such as these have on the interpretation of the inheritance of similar characters based on a comparison of constants derived from small parental and F_1 populations with very large F_2 populations. The fact that is decidedly significant in connection with the study of the inheritance of flower size is that the distributions of means when different parts of the total population are measured at different times is so strikingly different from the truly representative distribution derived from the measurement of all the plants throughout the whole season.

In Tables II to V we are concerned with the flower size of three of the semi-sterile hybrids of *N. Tabacum* varieties and *N. sylvestris* which have been mentioned in previous communications (cf. Goodspeed, 1913, 2). Tables II*a* and II*b* have to do with the means of spread and length of flowers on the plants of F_1 H_{137}. The facts brought out in these tables emphasize those already demonstrated by

TABLE II*a*

Frequency Distributions for Spread of Corollas in 13 F_1 H_{137}

Designation	Class Centers in Millimeters										No.	Mean	Coef. Var.
	30	31	32	33	34	35	36	37	38	39			
Means for totals...........					1	4	4				9	35.33±.15	1.89±.30
Means for first dates.......									6	3	9	38.33±.11	1.23±.20
Means for mid dates........				1	1		1	3	1	2	9	36.67±.44	5.30±.84
Means for last dates.......	1	4	2	1	1						9	31.67±.26	3.65±.58

TABLE II*b*

Frequency Distributions for Length of Corolla in 13 F_1 H_{137}

Designation	Class Centers in Millimeters							No.	Mean	Coef. Var.
	51	52	53	54	55	56	57			
Means for totals...........				1	4	4		9	55.33±.11	1.21±.19
Means for first dates........						7	2	9	56.22±.09	.74±.12
Means for mid dates........				3	3	3		9	55.00±.18	1.49±.24
Means for last dates........	1		4	3		1		9	53.44±.28	2.36±.37

Tables I*a* and I*b*. For spread of corolla, the range shown in the distribution designated "means for totals" is only 3 mm., from 34 to 36 mm. The range of fluctuation of flowers was 17 mm., from 26 to 42 mm. and an average of about 70 flowers was measured on each plant during a period of less than six weeks from August 9 to September 19. The difference between the spread of flowers on plants at the beginning of the flowering season, at the middle of the season, and towards its end is very striking. The results show a decrease in size from 38.33 ± .11 mm. through 36.67 ± .44 mm. to 31.67 ± .26 mm. in less than six weeks. With reference to length of corolla this progressive decrease in size is not nearly so striking, the decrease being from 56.22 ± .09 mm. through 55.00 ± .18 mm. to 53.44 ± .28 mm. The number of plants measured in this and in other cases is very small, but the fact that the measurements refer to the same plants gives them an added importance which compensates for this defect. No attempt was made to arrange the individual means into a distribution of "means for different dates"; but it is readily apparent from the data given that such an arrangement would have shown a more notable proportionate increase in the coefficient of variability than was shown in 13 22/07.

As was above noted H_{38}, the cross *N. Tabacum* var. *macrophylla* (U. C. B. G. 22/07) ♀ × *N. sylvestris* (U. C. B. G. 107/01) ♂, was made in 1911 and flowers were first measured in 1912. Tables III*a* and III*b* show the size of flowers borne by plants from a second sowing of the 1911 seed which flowered in 1913. The number of plants is again small but as explained above this fact should not detract from the significance of the data here reported. The period of measurement here was greater than in the previous cases, from August 4 to November 7, slightly over 3 months; and of the number of measurements taken,

Table III*a*

Frequency Distributions for Spread of Corollas in 13(11) F_1 H_{38}

Designation	Class Centers in Millimeters											No.	Mean	Coef. Var.
	31	32	33	34	35	36	37	38	39	40	41			
Means for totals				2	6	2						10	35.00±.13	1.81±.27
Means for first dates						1		2	3	2	2	10	39.10±.31	3.70±.56
Means for mid dates			2	1		2	2	2	1			10	36.10±.43	5.60±.85
Means for last dates	1	2	2	5								10	33.10±.22	3.15±.48

an average of over 80 for each plant, about half were taken on the last date of measurement. Here we have for spread the distribution

TABLE IIIb

Frequency Distributions for Length of Corolla in 13(11) F_1 H_{38}

Designation	Class Centers in Millimeters								No.	Mean	Coef. Var.
	51	52	53	54	55	56	57	58			
Means for totals..........			4	4	2				10	53.80±.16	1.39±.21
Means for first dates.....					1	2	2	5	10	57.10±.22	1.83±.28
Means for mid dates.....		1	4		3	2			10	54.10±.29	2.54±.38
Means for last dates......	3	2	2	2	1				10	52.60±.29	2.58±.39

of the "means for totals" showing a range of but 3 mm., from 34 to 36 mm.; although the range of flower size on these plants amounts to 21 mm., from 23 to 43 mm. The mean of the "means for totals" distribution is 35.00 ± .13 mm. as contrasted with 39.10 ± .31 mm. for the "first dates," 36.10 ± .43 mm. for the "mid dates," and 33.10 ± .22 mm. for the last dates. This means for the spread of corolla a progressive decrease of 6 mm. in two steps within the period of measurement. For the flowers of individual plants we have in some cases a greater decrease in mean flower size during the period. Thus in Plant 4 there is a decrease in flower size of 8.11 mm., from 40.00 to 31.89 mm., within one month, and in the distribution of means for some of the other individual plants we have nearly as great a decrease in size during the period of measurement. The measurements on length of corolla show, in a lesser degree, the same situation. Within the period of measurement, the lengths decreased 4.50 mm. and the measurements on some of the individual plants on different dates show a correspondingly larger decrease in length. Thus for Plant 4 the decrease was 6.44 mm., from 58.00 to 51.56 mm. during one month. The increase in the coefficient of variability during the period of measurement is again very marked and would obviously be still more so, if the system of arrangement of "means for different dates" followed in Tables I*a* and I*b* had been carried out.

Tables IV*a*, IV*b*, V*a*, and V*b*, have to do with two F_1 hybrids made between *N. Tabacum* varieties and *N. sylvestris*. 12 F_1 H_{38} and 13 F_1 H_{38} described in Tables IV*a* and IV*b*, do not differ genetically from 13 (11) F_1 H_{38} with which Tables III*a* and III*b* are concerned. They represent measurements on flowers of the plants from the first sowing of the H_{38} hybrid seed under the designation 1912, and on these same plants of the first sowing cut back and blooming the following year on their own roots under the 1913 designations. In 1912 the 14 or 15 flowers measured on each of the 25 plants of F_1 H_{38} were all measured on the same date late in the season, on October 31. These plants began to go out of flower during the last week of December and were cut back early in February. They soon put out new laterals which began to bear flowers early in May. In Tables IV*a* and IV*b* are given the distributions of means for spread and length of 21 of these plants. The remaining four of the original plants were lifted into pots and subsequently flowered in the greenhouse. The significance of the designation "means for totals of same plants 1912" is thus apparent. Tables V*a* and V*b* are arranged in exactly the same manner as Tables IV*a* and IV*b*. Twenty-four plants were originally measured

TABLE IV*a*

Frequency Distributions of Spread of Corolla in F_1 H_{38}

Designation	Class Centers in Millimeters												No.	Mean	Coef. Var.
	33	34	35	36	37	38	39	40	41	42	43	44			
Means for totals, 1913		1			2	5	1	4	3	4	1		21	39.57±.32	5.49±.57
Means for totals of same plants, 1912		2	6	8	4	1							21	35.81±.15	2.81±.29
Means for totals, 1912		2	7	11	4	1							25	35.80±.13	2.62±.25
Means for first dates, 1913			3		3	3	1	6	4	1			21	38.81±.31	5.43±.57
Means for mid dates, 1913	1		1	4	1		3	2	5	3	1		21	39.05±.40	7.00±.73
Means for last dates, 1913				1	3	2	4	2	2	3	2	2	21	40.05±.35	5.94±.62
Means for different dates, 1913				2	2	3	3	2	4	5			21	39.57±.30	5.09±.53

TABLE IV*b*

Frequency Distributions of Length of Corolla in F_1 H_{38}.

Designation	Class Centers in Millimeters									No.	Mean	Coef. Var.
	49	50	51	52	53	54	55	56	57			
Means for totals, 1913				2	2	3	10	4		21	54.57±.17	2.16±.22
Means for totals of same plants, 1912			1	8	5	5	2			21	52.95±.16	2.06±.21
Means for totals, 1912			1	12	5	5	2			25	52.80±.14	2.00±.19
Means for first dates, 1913					2	2	8	8	1	21	55.19±.15	1.82±.19
Means for mid dates, 1913					1	2	8	7	3	21	55.43±.15	1.81±.19
Means for last dates, 1913	1	1		1	2	8	7	1		21	53.81±.24	3.07±.32
Means for different dates, 1913	1			1	1	2	8	5	3	21	54.90±.26	3.27±.34

TABLE V*a*

Frequency Distributions for Spread of Corolla of F_1 H_{44}

Designation	Class Centers in Millimeters																						No.	Mean	Coef. Var.
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46			
Means for totals, 1913									2		2		1	2	1	1	4	2	3	1			19	39.42±.52	8.53± .93
Means for totals of same plants, 1912				2		2	2	1	2	4	3	1	1		1								19	33.11±.44	8.59± .94
Means for totals, 1912	1		1	3		3	2	1	2	4	3	1	1	1	1								24	32.38±.49	10.99±1.07
Means for first dates, 1913										2	2		3		2	5	5						19	38.53±.38	6.38± .70
Means for mid dates, 1913												3	1	2	2	2	3	2		3	1		19	40.16±.44	7.01± .77
Means for last dates, 1913						1		1		1	1	1		1	2	2	2	4		2		1	19	39.32±.64	10.51±1.15
Means for different dates, 1913						1		1		1		1			3	7	1			4			19	39.26±.59	9.67±1.06

in 1912, and five of them were transferred to the greenhouse when the plants were cut back. F_1 H_{38} and F_1 H_{44} were cut back on approximately the same dates.

TABLE V*b*

Frequency Distributions for Length of Corolla in F_1 H_{44}

Designation	Class Centers in Millimeters														No.	Mean	Coef. Var.
	46	47	48	49	50	51	52	53	54	55	56	57	58	59			
Means for totals, 1913....			1	1	1		2	5	1	3	5				19	53.42±.37	4.44±.49
Means for totals of same plants, 1912..........	2			2	1	1	5	1	3	2	1			1	19	52.16±.46	6.07±.66
Means for totals, 1912....	2			2	1	2	6	1	3	3	3			1	24	52.54±.42	5.84±.57
Means for first dates, 1913					1	1	2	1	4	8	1	1			19	54.05±.26	3.15±.34
Means for mid dates, 1913					1		1	4	3	2	6	2			19	54.53±.28	3.33±.36
Means for last dates, 1913	1	2			1		3	2	3	3	3	1			19	52.84±.49	5.99±.66
Means for different dates, 1913................	1	1			1		3	1	3	6	3				19	53.26±.43	5.27±.58

The 1913 measurements reported in these tables were taken with a different purpose in view and without a proper appreciation of the variation in flower size which has been shown in the previous tables. The measurements in this case were taken as the plants were just coming into flower. The method of procedure was to measure all the fully open flowers which were shedding pollen on the dates on which measurements were taken. In this way measurements were taken beginning at the first plant and working through the whole series one after the other. This process was repeated several times, the object being to obtain at least 100 measurements on each plant. As soon as this number had been exceeded on some date for some individual plant, no further measurements were taken on that particular plant during the period. Accordingly the measurements on these plants cover a rather short period from less than 3 weeks to slightly over 9 weeks, and represent a period during which the plants were coming into full flower as shown by the progressive increase in the number of flowers measured on individual plants with the advancement of the season, from as low as 4 at the beginning of the period to as high as 186 on one day at the end of the period. Naturally only a slight fluctuation in the mean would be expected during this period, particularly in view of the semi-sterile nature of the plants involved, and this is exactly the result obtained. That these plants, however, can show a wide variation in such respects is plainly evident from a comparison with the distributions for 1912.

Turning now to a consideration of these data, we find the situation somewhat different from that in the preceding tables. For the spread of corolla, the size is greater at the end of the period than at the beginning. As shown in Table IV*a* flowers on the "mid dates" of measurements are larger than on the "first dates," but smaller than on the "last dates"—there is a progressive increase in corolla spread during the period of measurement. The differences in size in both cases for the three measurements are within 2 mm. and differ from the mean of the "means for totals" by a still lesser amount. On the other hand, with reference to length of corolla in both Tables IV*b* and V*b*, the means for the first, mid, and last dates show a slight increase followed by a more marked decrease. This point will be discussed in a different connection below.

TABLE VI

Influence of the Age of the Plant on Corolla Measurements

Designation	Mean for			Difference between		
	First Date	Mid Date	Last Date	First and Mid Date	Mid and Last Date	First and Last Date
$13^{22}/_{07}$:						
spread......	28.48±.09		24.19±.22			−4.29±.24
length.......	45.60±.07		42.25±.15			−3.35±.17
13 F$_1$ H$_{137}$:						
spread......	38.33±.11	36.67±.44	31.67±.26	−1.66±.45	−5.00±.51	−6.66±.28
length.......	56.22±.09	55.00±.18	53.44±.28	−1.22±.21	−1.56±.34	−2.78±.30
13(11) F$_1$ H$_{38}$:						
spread......	39.10±.31	36.10±.43	33.10±.22	−3.00±.53	−3.00±.49	−6.00±.38
length......	57.10±.22	54.10±.29	52.60±.29	−3.00±.37	−1.50±.41	−4.50±.37
13 F$_1$ H$_{38}$:						
spread......	38.81±.31	39.05±.40	40.05±.35	+0.24±.51	+1.00±.53	+1.24±.47
length.......	55.19±.15	55.43±.15	53.81±.24	+0.24±.21	−1.62±.28	−1.38±.28
13 F$_1$ H$_{44}$:						
spread......	38.53±.38	40.16±.44	39.32±.64	+1.63±.58	− .84±.77	− .79±.74
length.......	54.05±.26	54.53±.28	52.84±.49	+0.48±.39	−1.69±.57	−1.21±.56

In Table VI the situation with reference to the differences in size of flowers borne on a plant at the beginning, toward the middle, and near the end of the flowering season or the period during which flowers were measured is summed up. This table is self-explanatory. The probable errors of all constants have been included. These differences should be significant if they are at least three times as great as their probable errors. This requirement is fulfilled in all cases except for 13 F$_1$ H$_{38}$ and 13 F$_1$ H$_{44}$, an explanation for which we have suggested above.

Under the general heading "age of plant factor" the question of the differences in size of flowers on plants growing the second year on their own roots and flowers on the same plants the first year is included. On this point the data is given in Tables IV*a*, IV*b*, V*a*, and V*b*, and in condensed form in Table VII. Two facts are strikingly evidenced by the results as given. First, the spread of corolla of flowers which are produced on new vigorous laterals from plants which have been given an involuntary period of rest by cutting back is distinctly larger than the spread of flowers measured toward the end of the first season of growth and flowering. These differences are distinct and significant when compared with their probable errors. The second significant point illustrated above has to do with the relatively slight difference between the length of corolla in the case of the two periods of measurement. A difference of 6.31 ± .68 mm. in the spread of F_1 H_{44} is associated with a difference of only 1.26 ± .61 mm. in the length of the same flowers and similarly for F_1 H_{38} we have a difference of 3.76 ± .35 mm. for spread as contrasted with 1.62 ± .24 for length of corolla.

TABLE VII

Influence of Different Years on Corolla Measurements on the Same Plants

Designation	Mean		Difference
	1912	1913	
F_1 H_{38}: spread	35.81±.15	39.57±.32	3.76±.35
length	52.95±.16	54.57±.16	1.62±.24
F_1 H_{44}: spread	33.11±.44	39.42±.52	6.31±.68
length	52.16±.46	53.42±.37	1.26±.61

It must be borne in mind in this connection that the plants the first year (12 F_1 H_{38} and 12 F_1 H_{44}) were measured during the last two weeks in October or toward the end of the period of measurement as given for the other groups of plants in 1913, while in 1913 (13 F_1 H_{38} and 13 F_1 H_{44}) the flowers were measured early in their second period of blooming. In other words, if in 1913 the flowers had been measured during October, their size would undoubtedly have been more nearly equal to that given by the measurements in 1912. The dates of measurement and results as they stand are, however, particularly significant for our purpose. There is obviously, also, a decided discrepancy in the number of measurements taken in 1912 as compared

TABLE VIII*a*

Frequency Distributions for Spread of Corolla of Individual Plants in F_1 H_{38},

Plant No.	Class Centers in Millimeters																										No.	Mean	Coef. Var.	$M_{1913}-M_{1912}$
	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49				
P_1, 1912										1		1	1	3	4	2	2										14	37.50±.33	4.91±.63	
1913											4	2	4	8	23	12	12	8	13	9	2	2	1				101	39.60±.18	6.77±.32	+2.10±.38
P_2, 1912									4	1	2	1	4		2	1											15	34.87±.40	6.61±.81	
1913							2			3	4	5	16	29	31	24	21	15	11	6	5	1					173	38.57±.13	6.67±.24	+3.70±.42
P_3, 1912								1	1	1	2	2	1	2	1	3											14	35.64±.46	7.16±.91	
1913														1	3	6	18	20	23	15	8	4	2	2			102	41.71±.13	4.60±.22	+6.07±.48
P_4, 1912											2	1	4	2	3	1	1	1									15	37.00±.34	5.32±.65	
1913									3	3	11	9	23	24	42	22	23	7	4								171	37.60±.11	5.64±.21	+0.60±.36
P_5, 1912									1		3	2	2	1	5		1										15	36.20±.37	5.81±.72	
1913										3	6	5	12	27	28	19	18	15	5	8				2			148	38.49±.14	6.49±.25	+2.29±.40
P_6, 1912									2	3	3		2	3	2												15	34.93±.36	5.96±.73	
1913					2			1	5	4	1	10	8	19	17	15	21	21	26	18	15	8	3	3			197	39.85±.17	9.00±.31	+4.92±.40
P_8, 1912									2		1	2	6	2	1	1											15	35.67±.32	5.19±.64	
1913												1	4	6	7	11	16	13	13	11	14	8	5	3			112	41.38±.18	6.78±.31	+5.71±.37
P_9, 1912												1	3	1	3	3	4										15	38.07±.29	4.34±.53	
1913															3	4	6	10	17	11	13	5	5	1	1		76	42.49±.17	5.04±.28	+4.42±.34
P_{10}, 1912											2	1	6	1	5												15	36.40±.24	3.73±.46	
1913															2	1	10	10	11	4	8	6	5	4		1	62	42.71±.21	5.79±.35	+6.31±.32
P_{11}, 1912									1		4	4	2	2	1	1											15	35.40±.30	4.93±.61	
1913							2	2	1		1		2	3	5	7	20	16	12	11	4	3	3	1			93	40.46±.23	8.06±.40	+5.06±.38
P_{13}, 1912										2		6	5	2													15	35.33±.20	3.21±.40	
1913														1	4	10	10	26	12	12	6	2	3				86	42.35±.14	4.57±.23	+7.02±.24
P_{14}, 1912								2		2	3	3	3	2													15	34.47±.32	5.28±.65	
1913														1	1	8	6	17	17	24	17	7	3	1			102	42.33±.13	4.50±.21	+7.86±.35
P_{15}, 1912										1	1	2	3	2	1	2	2	1									15	37.07±.40	6.19±.76	
1913													1	2	6	5	13	10	5	5	5	1	1				54	40.72±.20	5.29±.34	+3.65±.45
P_{16}, 1912									1	1	4	6	1	2													15	34.73±.22	3.71±.46	
1913									1	1	1	1	1	6	13	13	25	20	12	9	4	3	3				113	40.29±.16	6.16±.28	+5.56±.27
P_{17}, 1912									4	2	6	2	1														15	33.60±.21	3.57±.44	
1913										1	2	2	4	6	7	11	18	19	16	18	10	4	3	3	2	1	127	41.11±.18	7.29±.31	+7.51±.28
P_{18}, 1912							2		2	3	3	3			2												15	33.73±.39	6.71±.83	
1913										1	3	9	11	11	14	20	15	14	4	1							103	38.35±.15	5.70±.27	+4.62±.42
P_{19}, 1912								1	1	2	3	1	3	2	1												15	34.73±.33	5.51±.68	
1913	2	2	1	1	2	2	1	1	2	5	6	12	7	14	11	9	11	5	2								96	36.01±.28	11.21±.55	+1.28±.43
P_{20}, 1912											2	3	3	5	2												15	36.13±.22	3.48±.43	
1913				1					1		3	8	18	17	23	7	12	3	3	6	1	5					108	38.16±.19	7.67±.35	+2.03±.29
P_{21}, 1912									1	4	3	3	1	3													15	34.53±.28	4.59±.57	
1913									1	3	2	1	1	8	6	1	1	1									25	36.56±.30	6.15±.59	+2.03±.41
P_{22}, 1912									1		2	6	1	2	2	1											15	35.67±.31	4.98±.61	
1913									1	7	6	10	10	26	11	14	13	10	5								113	37.55±.16	6.51±.29	+1.88±.35
P_{25}, 1912											1	1	5	4	1	2	1										15	36.87±.27	4.19±.52	
1913	1	1		1	3	1	2	5	3	1	5	7	4	5	8	4		1	2								54	34.48±.38	11.93±.77	−2.39±.47

TABLE VIII*b*

Frequency Distributions for Length of Corolla in Individual Plants in F_1 H_{38}

Plant No.	Class Centers in Millimeters																										No.	Mean	Coef. Var.	$M_{1913}–M_{1912}$
	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59				
P_1, 1912																			1	3	4	3	3				14	54.29±.22	2.25±.29	
1913																1		3	4	7	14	30	30	11	1		101	54.97±.10	2.83±.13	+0.68±.24
P_2, 1912																1	3	2	2	2	3	2					15	52.20±.33	3.65±.45	
1913												1	1		1	2	5	3	5	7	13	53	46	29	7		173	55.05±.11	3.87±.14	+2.85±.35
P_3, 1912																1		3	1	3	3	2	1				14	52.93±.34	3.53±.45	
1913															1	1		3	3	10	12	41	20	9	2		102	54.78±.11	3.03±.14	+1.85±.36
P_4, 1912																			1	2	2	5	4	1			15	54.80±.23	2.42±.30	
1913															1	1	4	1	4	6	18	50	45	36	5		171	55.31±.09	3.08±.11	+0.51±.25
P_5, 1912																1		3	1	2	5		3				15	53.20±.35	3.77±.46	
1913																	2	3	7	17	38	42	32	7			148	54.53±.08	2.59±.10	+1.33±.36
P_6, 1912																3		3	5	1	3						15	51.67±.29	3.21±.40	
1913				2		1		1			1	5	3	6	7	11	9	11	13	19	23	39	32	12	2		197	52.75±.18	7.15±.24	+1.08±.34
P_8, 1912																1		1	6	3	4						15	52.47±.23	2.50±.31	
1913																1			1	4	9	22	25	31	17	2	112	56.07±.10	2.80±.13	+3.60±.25
P_9, 1912																			1	4	4	5		1			15	54.13±.21	2.22±.27	
1913													1		1		1		3	2	16	30	18	2	2		76	54.71±.14	3.27±.18	+0.58±.25
P_{10}, 1912																			4		5	4	2				15	54.00±.24	2.53±.31	
1913																		1	1	1	4	15	24	8	6	2	62	55.85±.12	2.59±.16	+1.85±.27
P_{11}, 1912																1	1	5	2	1	2	3					15	52.27±.33	3.59±.44	
1913															1	1	4	2	2	8	9	25	28	12	1		93	54.82±.14	3.58±.18	+2.55±.36
P_{13}, 1912																2	1	2	1	4	4	1					15	52.33±.32	3.54±.44	
1913																					3	21	32	18	12		86	56.17±.08	1.89±.10	+3.84±.33
P_{14}, 1912															1		1	2	1	2	4	4					15	52.93±.36	3.87±.48	
1913																	1	1		5	9	26	35	19	4	2	102	55.64±.10	2.56±.12	+2.71±.37
P_{15}, 1912																1				3	5	3	2		1		15	54.20±.33	3.51±.43	
1913											1		1				1	1	2	7	13	10	10	6	2		54	54.37±.23	4.57±.30	+0.17±.40
P_{16}, 1912																	2	2	4	3	4						15	52.33±.24	2.58±.32	
1913													1		2		1	4	5	10	16	30	29	10	5		113	54.73±.13	3.75±.17	+2.40±.27
P_{17}, 1912																1	4	3	5	2							15	51.20±.20	2.28±.28	
1913																1	2	1	2	10	17	34	39	17	4		127	55.17±.09	2.86±.12	+3.97±.22
P_{18}, 1912																1	3	2	5	2	2						15	51.67±.25	2.80±.34	
1913													1			3	3	2	3	13	27	30	19	2			103	54.14±.12	3.42±.16	+2.47±.28
P_{19}, 1912																	2	1	2	6	2	2					15	52.73±.26	2.81±.35	
1913	1	1		3	2	1	1	1	1	1	2	3	1	3	4	2	2	4	7	7	9	19	14	6	1		96	51.20±.41	11.52±.56	−1.53±.49
P_{20}, 1912																		1			7	1	6				15	54.67±.24	2.47±.30	
1913												1					1	2	1	8	13	25	27	23	6	1	108	55.37±.12	3.41±.16	+0.70±.27
P_{21}, 1912																	2	3	6	2	2						15	51.93±.21	2.28±.28	
1913															1		4		2	5	3	7	2	1			25	53.32±.30	4.20±.40	+1.39±.37
P_{22}, 1912																		1		5	7	2					15	53.60±.17	1.78±.22	
1913														4	1	2	2	2	11	10	17	20	23	15	6		113	54.40±.16	4.61±.21	+0.80±.23
P_{23}, 1912																		2		8	3	1	1				15	53.27±.22	2.32±.29	
1913									1				4	2	1	3	4	7	3	4	6	11	5	2	1		54	52.24±.32	6.64±.43	−1.03±.39

TABLE IX*a*

Frequency Distributions for Spread of Corollas in Individual Plants in F_1 H_{44}

Plant No.	Class Centers in Millimeters																												No.	Mean	Coef. Var.	$M_{1913}-M_{1912}$
	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49				
P_2, 1912													7	4	3	1	2												17	35.24±.22	3.84±.44	
1913									1	1	1	1	3	2	1	1	6	10	15	13	24	27	12	14	6	2			140	41.56±.18	7.55±.30	+ 6.32±.28
P_3, 1912					1	5	5	2	1	1																			15	28.00±.22	4.52±.56	
1913							2				1	2	4	3	6	7	20	21	21	15	11	3	2	1					119	38.89±.17	7.17±.31	+10.89±.28
P_5, 1912						1	3	3	4	2	2																		15	29.60±.25	4.91±.60	
1913														1	1	7	4	12	20	28	29	26	16	4	4	1			153	41.50±.12	5.21±.20	+11.90±.28
P_7, 1912												2	7	3	3														15	34.47±.18	2.97±.37	
1913															2	3	1	6	17	19	30	39	36	30	21	12	1	1	218	43.17±.11	5.38±.17	+ 8.70±.21
P_9, 1912										1	1	2	3	3	4	1													15	34.47±.28	4.72±.58	
1913													1	1	4	9	15	23	45	37	38	18	14	2					207	40.61±.09	4.96±.16	+ 6.14±.29
P_{10}, 1912										1	2	4	3	3	2														15	33.73±.25	4.26±.52	
1913							1				1	2			2	5	11	18	30	24	18	5	1						118	39.85±.14	5.66±.25	+ 6.12±.29
P_{11}, 1912									1	3	5	1	2	3															15	32.60±.28	4.86±.60	
1913		1		3		2	3	1	5	7	13	18	19	23	27	24	24	12	13	6		1							202	35.36±.16	9.51±.32	+ 2.76±.32
P_{12}, 1912											1	3	4	4	2	1													15	34.40±.23	3.80±.47	
1913				1												2	1	2	4	8	7	10	20	16	24	20	6	5	126	44.48±.19	6.95±.30	+10.08±.30
P_{13}, 1912												1	4	2	2	3	3												15	35.73±.29	4.62±.57	
1913													1		1	1		4	10	15	22	32	37	23	13	2	1		162	43.09±.11	4.84±.18	+ 7.36±.31
P_{14}, 1912									2	1	3	3	4	1	1														15	32.87±.29	5.08±.62	
1913									1				1		3	7	9	30	38	30	28	19	10		1				177	40.52±.11	5.16±.19	+ 7.65±.31
P_{15}, 1912								3	5		2	2	2	1															15	31.33±.35	6.35±.78	
1913												1				4	7	20	10	17	24	26	25	18	3	1			156	41.96±.13	5.70±.22	+10.63±.37
P_{17}, 1912												1	4	5	3	1	1												15	35.13±.22	3.58±.44	
1913				2		2	1	1	9	1	14	14	23	29	33	57	48	41	54	33	11	4	4						381	37.30±.11	8.52±.21	+ 2.17±.25
P_{18}, 1912													1	2	3	4	2		3										15	37.07±.31	4.87±.60	
1913				1	1		1	1				2	6	7	9	6	9	10	15	13	19	23	14	23	14	10	4		188	41.37±.21	10.29±.36	+ 4.30±.36
P_{19}, 1912						3	1	2	4	4	1																		15	29.53±.28	5.37±.66	
1913		2	2	2	5	6	8	8	4	12	21	19	27	20	15	8	9	2	1										171	32.73±.18	10.68±.39	+ 3.20±.33
P_{20}, 1912					1	4	5	2	2		1																		15	28.27±.26	5.24±.65	
1913				2		3	2	5	15	14	20	22	35	36	33	37	23	13	14	6	2								282	34.96±.13	9.06±.26	+ 6.69±.29
P_{22}, 1912															2	2	3	1	2	1	1	2	1						15	39.47±.44	6.47±.80	
1913											1	2		1	5	5	6	4	14	12	27	17	21	21	16	7	4	3	166	42.60±.17	7.66±.28	+ 3.13±.47
P_{23}, 1912									2	3	5	2	2	1															15	32.13±.25	4.38±.54	
1913													2	1	7	4	12	14	9	2									51	38.18±.16	4.33±.29	+ 6.05±.30
P_{24}, 1912						1	1	3	3	3	1	1	1	1															15	30.60±.37	6.94±.85	
1913	2	2	3	3		3	6	6	12	8	10	9	10	16	14	16	11	3	2										136	33.06±.24	12.49±.51	+ 2.46±.44
P_{25}, 1912										1	1	2	3	3	3	2													15	34.53±.40	4.95±.61	
1913													1	8	4	14	26	25	17	3	3								101	38.30±.11	4.33±.21	+ 3.77±.41

TABLE IX*b*

Frequency Distributions for Length of Corolla in Individual Plants in F_1 H_{44}

Plant No.	Class Centers in Millimeters																														No.	Mean	Coef. Var.	$M_{1913}-M_{1912}$
	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63				
P_2, 1912																		1	1	3	4	6	2								17	54.12±.22	2.45±.28	
1913													1			1	2	1	6	15	32	42	19	17	4						140	54.69±.10	3.23±.13	+0.57±.24
P_3, 1912																			3	1	3	3		5							15	54.73±.33	3.43±.42	
1913			1			1				1			1	2	2	2	4	3	12	19	24	21	16	6	2	2					119	53.44±.20	6.17±.27	−1.29±.38
P_5, 1912																			1		3	2	6	2	1						15	55.47±.25	2.62±.32	
1913																2	9	1	9	12	23	29	33	20	10	5					153	54.92±.12	4.02±.16	−0.55±.28
P_7, 1912																1	5	6	3												15	50.73±.18	1.52±.19	
1913								1								3	2	26	40	58	55	27	6								218	53.02±.08	3.12±.10	+2.29±.20
P_9, 1912																	1	1	1	3	4	2	3								15	53.73±.30	3.22±.40	
1913														1	1	1		1	4	5	17	48	66	39	22	2					207	55.74±.08	2.99±.10	+2.01±.31
P_{10}, 1912																		1	1	3	7	2	1								15	53.73±.21	2.20±.27	
1913												1			2		1	3	4	9	12	34	36	12	4						118	54.91±.12	3.65±.16	+1.18±.24
P_{11}, 1912																						1	2		2	2	2	4	1	1	15	59.33±.40	3.83±.47	
1913								1				4	2	2	4	4	5	6	13	10	10	17	19	26	15	22	25	11	6		202	55.89±.19	7.32±.24	−3.44±.44
P_{12}, 1912																					2	3	7	3							15	55.73±.16	1.67±.21	
1913						1							1		1	2	1	2	1	11	6	21	31	23	21	3	1				126	55.55±.16	4.87±.21	−0.18±.23
P_{13}, 1912																	2	1	4	7	1										15	52.27±.20	2.15±.26	
1913											1				3	5	12	22	48	37	27	6	1								162	52.20±.09	3.14±.12	−0.07±.22
P_{14}, 1912															2		2		5	4	1	1									15	51.80±.34	3.74±.46	
1913									1				1	1	3		9	19	22	27	56	20	17	1							177	53.14±.11	3.90±.14	+1.34±.36
P_{15}, 1912																				3	5	3	4								15	54.53±.19	1.99±.25	
1913																5	3	7	8	22	46	37	19	3	4	1		1			156	54.10±.10	3.52±.13	−0.43±.21
P_{17}, 1912														1	5		5	4													15	49.40±.29	3.49±.42	
1913		1				1	2	7	3	8	14	14	15	24	41	33	60	64	48	27	16	3									381	49.29±.11	6.45±.16	−0.11±.31
P_{18}, 1912																2	2	3	1	3	3		1								15	52.00±.35	3.85±.47	
1913	1				1	1	1	3	2	2		4	3	7	9	12	19	9	18	15	8	9	18	22	17	6		1			188	52.35±.24	9.14±.32	+0.35±.42
P_{19}, 1912												4	6	2	3																15	46.27±.19	2.30±.28	
1913			2	1	1		2	6	10	7	12	13	9	11	7	17	34	17	16	4	2										171	47.52±.20	8.07±.29	+1.25±.28
P_{20}, 1912													1				1	1	4	4	3	1									15	52.27±.36	3.98±.49	
1913					1	1	1	1		2	3	9	10	8	6	10	11	23	13	27	17	37	37	28	20	10	7				282	53.25±.17	8.05±.23	+0.98±.40
P_{22}, 1912																		2	6	1	2	4									15	53.00±.25	2.76±.34	
1913													1	1	4	1		3	4	6	9	35	43	25	23	10	1				166	55.67±.13	4.31±.16	+2.67±.28
P_{23}, 1912							1							3	3	7		2													15	48.67±.21	2.45±.30	
1913															1	2	2	4	5	11	14	10	1	1							51	53.18±.17	3.47±.23	+4.51±.27
P_{24}, 1912														2		3	1	6	2	1											15	50.27±.29	3.37±.41	
1913	1					2	3	4	2	1	1	6	6	7	8	8	7	15	13	25	12	9	4	2							136	50.11±.25	8.76±.36	−0.16±.38
P_{25}, 1912																	3	3	2	3	3	1									15	52.20±.28	3.07±.38	
1913																			1	4	17	23	31	19	5	1					101	55.59±.09	2.36±.11	+3.39±.29

with the number taken in 1913. The first set of measurements was, however, taken with unusual care, since it was in both cases desired to measure all the plants on the same day, and since it was recognized at the time that to do so only a few measurements could be taken. When the measurements were made the terminal inflorescences were practically out of flower and only the largest flowers and those shedding pollen, on the flowering laterals were measured. The method of taking measurements in 1913 has already been described.

In Tables VIII*a*, VIII*b*, IX*a*, and IX*b* are given the distribution for individual plants of F_1 H_{38} and F_1 H_{44} in 1912 and the same plants cut back and flowering in 1913. In the last column is given the differences between the means and the probable errors of these differences.

As a third consideration under the general heading "age of plant factor," we have data bearing on the influence on flower size of the presence of developing seed capsules following the earlier flowers of the plant. In the earlier experiments on the inheritance of flower size in Nicotiana (cf. Goodpseed, 1912) the effort was made to measure every fully developed flower each day on all the plants under consideration. In such a case there are at the end of the period of measurement no developing or matured seed capsules on the plant. It was early observed that when these plants, from which all the flowers had been picked off for a month or more, were allowed to make seed normally the flowers they bore among the developing seed capsules were distinctly smaller than those previously measured. When this particular point was investigated in 1913, the observations previously made were strikingly confirmed and the distinct relation between the longevity of the plant and the amount of seed allowed to mature was further evidenced.

Figures 1 and 2 bear out the above statement without room for doubt or question. Figure 1 shows two plants of 13 22/07; Plant 14 on the right and Plant 15 on the left in the photograph. The photograph was taken 2 weeks after the last measurement on Plant 15. In the case of Plant 15 the flowers too old to be measured and developing seed capsules were picked off twice a week, while from Plant 14 only the 42 flowers measured were removed. Every fully developed flower present on Plant 15 on each of the dates of measurement was measured. In a month the spread of corolla of flowers on Plant 14 dropped from 27.38 mm. to 21.60 mm., or 5.78 mm., while during a similar period the spread of flowers on Plant 15 had decreased in size only 0.75 mm. Nearly a month later the corolla spread of Plant 15 had decreased 2.29 mm. while on Plant 1, which also was measured on September 22,

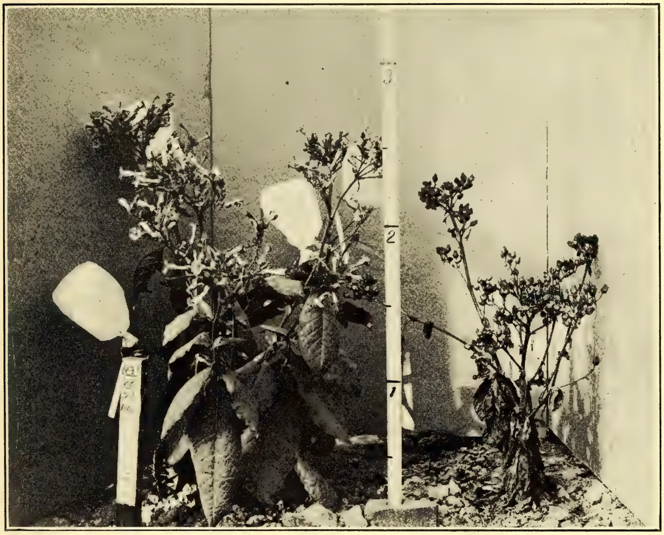

FIG. 1. Influence of removal of flowers on plants 13 22/07.

the decrease in spread was 5.40 mm. A somewhat corresponding situation holds for the length of corolla. Thus on Plant 14 the decrease in length was 4.55 mm. and on Plant 15 it was only 0.25 mm. Nearly a month later the decrease in the case of Plant 15 was 0.79 mm. and that of Plant 1 was 4.00 mm. Plant 15 continued to flower until the middle of December by which time the other 74 plants of 13 22/07 had been dead for over two months.

Figure 2 represents two F_2 plants of H_{103} which is a hybrid between two flower size varieties of *N. acuminata* (cf. Goodspeed, 1912). Tables X*a* and X*b* give the distribution of spread and length of corolla for Plants 13 and 14 of this hybrid, 13 F_2 H_{103} P_2. As will be seen these

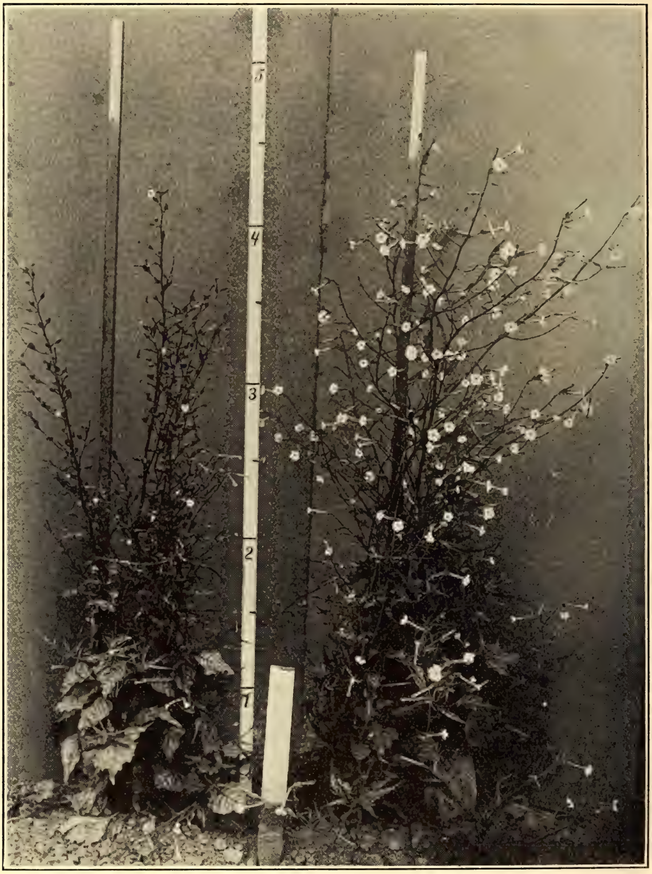

FIG. 2. Influence of removal of flowers on plants 13 $F_2H_{103}P_2$.

F_2 individuals were practically identical in spread and length of corolla on the first dates of measurement. They may, therefore, be compared with each other directly, so far as flower size is concerned. In the case of Plant 13, the old flowers and developing seed capsules were picked off, while Plant 14 was allowed to mature seed normally and only the 49 flowers measured were removed. The difference between the size of the few flowers present on Plant 14 and the numerous flowers on Plant 13, as seen in figure 2, is striking. Table X*a* shows that for spread of corolla in Plant 13 the decrease in size during the period of measurement was only 0.15 mm. as contrasted with a decrease of 6.50 mm. for the flowers of Plant 14. Similarly for length of corolla, Plant 13 showed a decrease of 1.16 mm. while Plant 14 showed a decrease of 5.17 mm.

TABLE X*a*

Influence of Removal of Flowers on Spread of Corolla in 13 F_2 H_{103} P_2

Plant No.	Date	Class Centers in Millimeters													No.	Mean
		14	15	16	17	18	19	20	21	22	23	24	25	26		
P_{13}....	August 22.....									3	4	3	2		13	23.23
	August 25.....							1		3	5	1	2		12	22.92
	September 9...				1	1		1	1	3	3	1		1	12	21.75
	September 20..							1	2	2	1	3	2	1	12	23.08
P_{14}....	August 22.....								1	2	3	2	2	2	12	23.67
	August 25.....								2	2	5	3	1		13	22.92
	September 9..					2	1	2	5	1	1				12	20.42
	September 20..	1	1	1	6		3								12	17.61

TABLE X*b*

Influence of Removal of Flowers on Length of Corolla in 13 F_2 H_{103} P_2

Plant No.	Date	Class Centers in Millimeters														No.	Mean
		28	29	30	31	32	33	34	35	36	37	38	39	40	41		
P_{13}...	August 22...								1	1	2	5	1	2	1	13	38.08
	August 25...						1	1	1	3		5		1		12	36.67
	September 9.					1				4	4	2		1		12	36.67
	September 20							3		1	4	2	2			12	36.67
P_{14}...	August 22...							1	1	6	2	2				12	36.25
	August 25...								3	3	3	3		1		13	36.77
	September 9.								3		1	5	3			12	37.42
	September 20	1	1	2	4	2	1		1							12	31.08

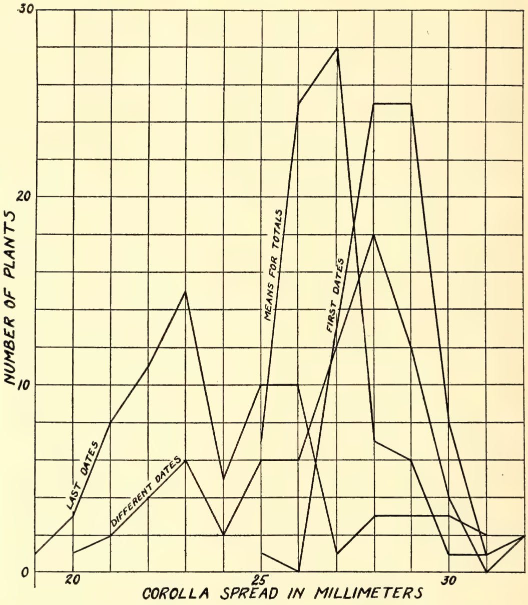

FIG. 3. Frequency polygons of variation for corolla spread of 13 22/07.

2. "*Age of Flower*" *Factor*

Under this heading, "'Age of Flower' Factor," we desire to illustrate two rather distinct sets of phenomena with reference to flower size in Nicotiana; first, the fact that at any given date throughout the flowering season the flowers on the terminal inflorescence differ in size from those borne on the laterals which come into flower late in the growing season, and second, the fact that with reference to apparently fully opened flowers, those with anthers shedding pollen are larger than flowers which are not shedding pollen. Tables XI*a*, XI*b*, XII*a*, XII*b*, XIII*a*, XIII*b*, XIV*a*, XIV*b*, XV*a*, and XV*b* illustrate these points.

TABLE XI*a*

Frequency Distributions for Corolla Spread of Flowers of 13(11) F_1 H_{38}

Time	Class Centers in Millimeters																				No.	Mean	Coef. Var.
	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43			
Before anthesis.......	1	2	5	4	8	7	10	8	10	13	12	19	17	20	10	2	3				151	33.37±.20	11.04±.43
After anthesis.......						2	3	3	6	6	15	7	12	14	34	48	52	19	3	1	225	37.90±.12	7.32±.23
Difference in means..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	....	4.53±.23	

TABLE XI*b*

Frequency Distribution for Tube Length of Flowers of 13(11) F_1 H_{38}

Time	Class Centers in Millimeters																		No.	Mean	Coef. Var.
	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60			
Before anthesis.........	2	2	3		5	5	2	9	4	11	6	10	18	35	23	16			151	54.01±.20	6.76±.26
After anthesis..........					1	2		5	1	18	15	13	30	53	42	29	14	2	225	55.63±.10	4.16±.13
Difference in means....	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	......	1.62±.22	

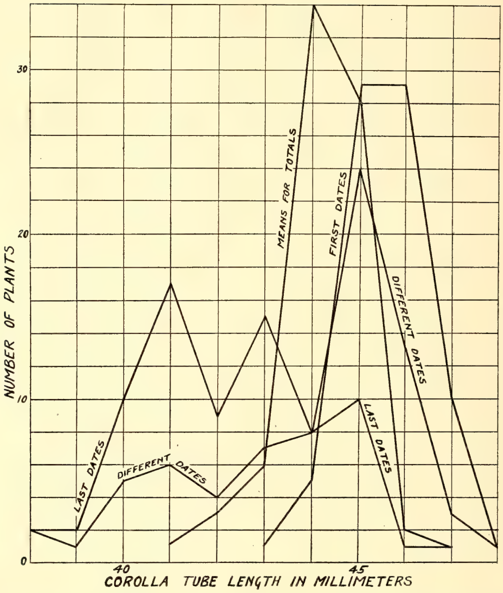

FIG. 4. Frequency polygons of variation for corolla tube length of 13 22/07.

With reference to the difference in size of fully opened flowers before and after the shedding of pollen the data is given in Tables XI*a*, XI*b*, XII*a*, and XII*b*. In these tables the flowers measured before shedding pollen, designated "before anthesis" in the tables, are compared with flowers measured on the same dates on the same plants after shedding pollen, designated "after anthesis" in the tables. In the case of 12 (11) F_1 H_{38}, the difference between the spread of "older" and "younger" flowers reaches more than 5 mm. for individual plants and averages 4.53 ± .23 mm. for the 10 plants measured. A corresponding condition does not hold for length of corolla where the maximum difference in size does not reach 3 mm. and averages only 1.62 ± .22 mm. For the "older" and "younger" flowers of 13 F_1 H_{137}, we have the same situation as shown in Tables XII*a* and XII*b*. The

TABLE XII*a*

Frequency Distribution for Corolla Spread of Flowers of 13 F_1 H_{137}

Time	Class Centers in Millimeters																	No.	Mean	Coef. Var.
	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42			
Before anthesis	1	5	5	5	13	9	21	30	22	20	14	13	4	1				163	33.13±.14	8.11±.30
After anthesis		3	6	13	11	16	15	14	14	24	27	66	69	58	33	14	1	384	36.31±.11	9.16±.22
Difference between means	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.....	3.18±.17	

TABLE XII*b*

Frequency Distribution for Tube Lengths of Flowers of 13 F_1 H_{137}

Time	Class Centers in Millimeters																	No.	Mean	Coef. Var.
	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60			
Before anthesis						1	2	2	2	9	14	33	46	35	19			163	55.68±.09	3.04±.11
After anthesis	1		1	1	3	1	6	11	15	32	28	76	104	75	27	2	1	384	55.21±.07	3.87±.09
Difference between means	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.....	.47±.11	

TABLE XIII*a*

Frequency Distribution for Corolla Spread of Flowers of 13(11) F_1 H_{38}

Position	Class Centers in Millimeters																			No.	Mean	Coef. Var.
	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41			
On laterals.............							2	3	3	6	6	15	7	12	11	12	13	13	3	106	36.14±.20	8.30±.38
On terminals............	2	1		5	7	2	7	10	4	5	5	3	10	7	10	4	4	1		87	32.40±.31	13.15±.67
Difference between means	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	....	3.74±.37	

TABLE XIII*b*

Frequency Distribution for Tube Length of Flowers of 13(11) $F_1 H_{38}$

Position	Class Centers in Millimeters																				No.	Mean	Coef. Var.
	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57			
On laterals..........										1	2		5	1	18	15	9	26	22	7	106	53.98±.14	3.92±.18
On terminals........	1			2		1	2	3	7	5	10	6	9	8	5	13	9	6			87	49.89±.26	7.27±.37
Difference between means	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	4.09±.30	

spread of the "younger" flowers averages 3.18 ± .17 mm. smaller than the spread of "older" flowers, while the length of "younger" flowers is slightly greater, .47 ± .11 mm., than that of the "older" flowers. This condition is undoubtedly due to the indirect method of comparison which we have here adopted and not to any actual shortening of the corolla tube. At the same time it renders even more significant the increase in corolla spread after anthesis which was found in measurements on the same flowers. This fact has been confirmed by direct measurements on a few individual flowers at these two stages in development. The length of time that intervenes between these two conditions in the flower is dependent to a considerable degree on weather conditions in the field, but in general occupies from 3 to 10 hours. We may, therefore, state that the spread of the flowers on these F_1 species hybrids increases distinctly within the period intervening between the opening back of the corolla limb and the dehiscence of the mature anthers, while, as might have been expected, the length of the corolla tube increases only slightly, if at all, during a corresponding period.

TABLE XIV*a*

Frequency Distribution for Corolla Spread of Flowers of 13 F_1 H_{137},

Position	Class Centers in Millimeters											No.	Mean	Coef. Var.
	31	32	33	34	35	36	37	38	39	40	41			
On laterals.					1	9	14	13	17	9	2	65	38.09 ± .12	3.71 ± .22
On terminals.	1	3	10	11	6	5	4	4	1			45	34.67 ± .19	5.47 ± .39
Difference between means	..	..	....	....	..	..	....	....	....	..	..	....	3.42 ± .22	

TABLE XIV*b*

Frequency Distribution for Tube Length of Flowers of 13 F_1 H_{137},

Position	Class Centers in Millimeters											No.	Mean	Coef. Var.
	47	48	49	50	51	52	53	54	55	56	57			
On laterals.							4	6	20	21	14	65	55.54 ± .09	2.00 ± .12
On terminals.	1	3		4	8	6	12	8	2	1		45	52.11 ± .20	3.78 ± .27
Difference between means .	..	..	..	..	..	..	....	..	....	....	....	....	3.43 ± .22	

The second point under the "age of flower" factor is illustrated in Tables XIII*a*, XIII*b*, XIV*a*, XIV*b*, XV*a*, and XV*b*. For 13 (11) F_1 H_{38} and 13 F_1 H_{137} the flowers produced on the terminal inflorescences are distinctly smaller than those borne by the laterals which began flowering late in the growing season. As in the previous cases these tables represent comparisons made between flowers measured on the same dates on the same plants. The difference in size here holds true for both spread and length of corolla. With reference to the data on this point given, for 13 22/07 in Tables XV*a* and XV*b* another interesting fact is brought out. The differences between the size of flowers borne on different parts of the plant correspond to those of the two F₁ hybrids, but the amount of difference is considerably less and the differences for spread and length of corolla do not correspond so closely. This is explained by the fact that the 20 plants upon which the measurements were taken were practically the only ones which had not gone almost entirely out of flower. As noted in the field records at the time, the inflorescences on the laterals were covered with developing seed capsules, while the flowers measured on the terminal inflorescences were produced from adventitious buds among dry seed capsules that had dehisced. Physiological conditions within the plant operating to throw the balance of the products of metabolic activity to the production of seed rather than to the formation of new, normally large flowers, are more equally operative in the case of both the terminal inflorescences and the lateral inflorescences of 13 22/07

TABLE XV*a*

Frequency Distribution for Corolla Spread of Flowers of $13^{22}/_{07}$

Position	Class Centers in Millimeters																	No.	Mean	Coef. Var.
	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35			
On laterals				8	18	25	42	48	53	41	47	48	25	21	4	1	2	383	27.55±.09	9.67±.24
On terminals	1	2	4	4	17	19	26	15	12	8	7	1						116	24.99±.14	8.67±.38
Difference between means	..		..	....	....	....	....	....	....	....	...	....	....	....	..	..	..	....	2.56±.17	....

TABLE XV*b*

Frequency Distribution for Tube Length of Flowers of $13^{22}/_{07}$

Position	Class Centers in Millimeters										No.	Mean	Coef. Var.
	38	39	40	41	42	43	44	45	46	47			
On laterals		1	11	24	37	84	99	94	27	6	383	43.70±.05	3.45±.08
On terminals	3	3	9	16	30	29	19	7			116	42.46±.10	3.60±.16
Difference between means	..	....	....	....	....	....	....	....	....	..	......	1.24±.11	....

than in the case of corresponding inflorescences of the two F_1 hybrids, which have a much longer growing season and which are almost completely sterile as compared with 12 22/07.

3. *Other Factors*

Under this heading we desire to present data which have to do with the differences in size between the flowers of plants the first year in the field and flowers on cuttings of these plants flowering in 6 inch pots in the greenhouse. Tables XVI*a* and XVI*b* give a condensed statement of the results of these measurements. The cuttings were taken during October, 1912, and came into flower in the greenhouse in April, 1913. The flowers were measured for the most part during the period from April 14 to May 12, although a few flowers were measured on some of the plants on July 28. The plants were given ordinary greenhouse treatment.

These tables bring out a rather surprising situation with reference to the flower size of the cuttings as compared with that of the original plants the first and second years. While the spread of the flowers on Plant 7 in 1913 was 8.70 ± .21 mm. larger than the spread of those measured on Plant 7 in 1912, on the other hand the spread of flowers on the three cuttings of Plant 7 lay about midway between these two means, and significantly different from either as shown in the last two columns of Table XVI*a*. In other words, the spread of flowers on the plant in the field during May 1913 and on the cuttings of this plant in the greenhouse on the same dates differed to the extent of nearly 4 mm. The length of flowers as shown in Table XVI*b* behaves differently under these same conditions. The flowers on Plant 7 in 1913 averaged only 2.29 ± .15 mm. larger than those of 1912 while for the cuttings the average length of corolla was 3.71 mm. greater than in 1912. For the plant in the field and the cuttings in the greenhouse flowering during the same period the length of corolla averaged 1.42 mm. greater in favor of the cuttings. We have then with reference to corolla spread an average flower size greater in the field than on the cuttings in the greenhouse and the reverse condition, but not so marked, in the case of corolla length.

During the past two years some 100 plants of *N. sylvestris* have been grown in groups under somewhat different field conditions and under exceedingly different greenhouse conditions. Fifty plants were grown in 1914 in a rather heavily manured, somewhat shaded, and

TABLE XVI*a*

Variation in Spread of Corolla in Different Years and under Different Conditions in $F_1\ H_{44}\ P_7$

Designation	Class Centers in Millimeters																	No.	Mean	Coef. Var.	$M_{1912}-M_c$	$M_{1913}-M_c$
	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49					
1912	2	7	3	3														15	34.47±.18	2.97±.37		
1913				2	3	1	6	17	19	30	39	36	30	21	12	1	1	218	43.17±.11	5.38±.17		
Cutting No. 1		1	2	3	9	9	15	21	10	1	1							72	38.99±.14	4.43±.25	−4.52±.23	+4.18±.18
" " 2			1	3	6	5	8	18	4	2								47	39.04±.16	4.16±.29	−4.57±.24	+4.13±.19
" " 3				1	3	4	9	8	10	2	1							38	39.66±.17	3.89±.30	−5.19±.25	+3.51±.20

TABLE XVI*b*

Variation in Length of Corollas in Different Years and under Different Conditions in $F_1\ H_{44}\ P_7$

Designation	Class Centers in Millimeters										No.	Mean	Coef. Var.	$M_{1912}-M_c$	$M_{1913}-M_c$
	49	50	51	52	53	54	55	56	57	58					
1912	1	5	6	3							15	50.73±.13	1.52±.19		
1913	3	3	26	40	58	55	27	6			218	53.02±.08	3.12±.10		
Cutting No. 1				3	13	14	24	16	2		72	54.60±.10	2.22±.12	−3.87±.16	−1.58±.13
" " 2			2	6	8	12	14	5			47	53.96±.16	2.48±.17	−3.23±.21	−0.94±.18
" " 3			1	1	3	10	14	5	3	1	38	54.76±.15	2.49±.19	−4.03±.20	−1.74±.17

moist garden plot, and an almost equal number some distance away on higher, unshaded, and unfertilized land. Eleven of these plants in the early rosette stages were transplanted with soil into pots and grown in the greenhouse. These eleven plants were treated with 3 applications of varying amounts of sodium nitrate, one pot receiving the maximum of 120 gm. and the amounts in the remaining pots decreasing in more or less regular ratio. Thus one pot received 3 gm.; one, 6 gm.; one, 10 gm.; one, 15 gm.; one, 30 gm.; one, 45 gm.; and so on.

No detailed measurements have been taken on the size of flowers on the treated plants in the greenhouse or on the plants grown under the contrasting field conditions. Inspection, actual comparison of flowers, and a few measurements, however, leave no doubt as to the relative size of flowers on the three groups of plants. The plants growing on the garden plot are in every way more vigorous and better developed as to vegetative characters than those on the exposed, unfertilized land. An off-hand inspection of the two groups of plants is convincing as to the fact that there is an increase in size of flowers more or less proportional to the increase in vegetative luxuriance. This fact is so very apparent that it scarcely needs the confirming evidence which is given by measurement and direct comparison of flowers from the two groups of plants. Spread of corolla is considerably larger in the flowers on the plants growing in the garden plot while the length of corolla is not correspondingly increased, though it, also, is greater.

With reference to the treated plants in the greenhouse the smaller amounts of nitrate scarcely serve to increase the vegetative vigor over that of the central plants. An increase in size of vegetative characters soon occurs, however, as the amount of nitrate is increased and after a certain point falls until near the maximum of application the plants are small and weak. These latter plants produce no flowers or only a few flowers that are strikingly diminutive in size as compared with the flowers on the central plants. As the amount of sodium nitrate is decreased until the optimum amount for vegetative growth is reached, the size of flowers increases distinctly, although the flowers on the treated plants that show maximum vegetative vigor are only slightly larger than those on the control plants. Such measurements as have been taken confirm the above statements. The briefest inspection of these 11 plants has served to convince observers that the flowers on the plants the vegetative growth of which has been restricted by too large applications of nitrate are smaller than the flowers of the plants that have been treated with smaller amounts.

IV. Discussion of Results

With reference to the question of the stability of the character complex, corolla size, under conditions attending development, the results of measurements of flowers on plants of Nicotiana as tabulated and discussed above seem pertinent. East (loc. cit., p. 178) illustrated his conception of the expression "conditions attending development" by mentioning the behavior of vegetative and floral characters in Nicotiana under favorable and unfavorable conditions of greenhouse culture, under poor field conditions and under field conditions at their best, and the case of cuttings versus the plants in the field from which they came. As a result of his observations, unsupported by the presentation of numerical data, East states, first, that tobacco plants growing on land where all conditions are at their best for maximum vegetative growth bear flowers of the same size as those borne by sister plants which are forced to come to maturity on land not so favorable for maximum vegetative growth. Similar observations in the case of our cultures do not confirm any such conclusion. Very large, vigorous plants growing in rather rich garden soil bear distinctly larger flowers than sister plants not so large or vigorous growing in poor soil. For example the flowers of one plant of *N. sylvestris* chosen at random from the more vigorous group averaged 43.12 mm. in spread and 84.12 mm. in length of corolla as compared with 37.40 mm. and 78.57 mm. for the average spread and length of the *most vigorous* plant among the group growing on the poorer soil. The largest flower to be found on the 50 plants of this latter group gave a spread of 42 mm. while over 20 flowers with a spread above 48 mm. were measured on only 10 plants of the former group.

In East's cultures, secondly, the flowers borne on plants in the field and on cuttings of these plants grown in small pots in the greenhouse are of the same size. The small amount of data available seems to show that rather a different condition of affairs is present in our cultures. Cuttings of a hybrid grown in pots in the greenhouse bore flowers the spread of which averaged nearly 4 mm. smaller than the spread of flowers on the plants in the field and over 1 mm. longer as to length of corolla. Cuttings of another F_1 hybrid, F_1 H_{38}, received little or no attention in the greenhouse and the average spread of their flowers was over 8 mm. smaller and the length of corolla over 2 mm. smaller than the spread and length respectively of flowers borne on plants in the field in 1912, and still smaller than the flowers of F_1 H_{38} in 1913.

With reference to the influence upon flower size of favorable and unfavorable greenhouse treatment our results are at variance with those of East. While in his cultures the size of flower did not vary under different conditions of greenhouse culture, our plants, under conditions which may be compared with good and poor greenhouse treatment, did show a distinct modification as to flower size as well as in respect to size of vegetative characters. In a series of pot experiments in which plants of *N. sylvestris* were treated with varying amounts of sodium nitrate, the plants receiving what seemed to be the optimum amount of salt produced larger flowers than the plants which received the maximum and minimum amounts of nitrate. Thus two plants which were treated with very nearly the same amounts of sodium nitrate showed an average difference in spread of about 3 mm. and about the same average difference in length. It is interesting in this connection to note that the plant treated with less nitrate produced flowers nearly 3 mm. smaller as to spread and over 2 mm. smaller as to length of corolla as compared with the flowers of plants of *N. sylvestris* grown on poorer land, and correspondingly smaller when compared with the size of flowers borne on plants grown in richer garden soil; *i. e.*, 8.12 mm. in spread and 12.28 mm. in length.

Now these variations in flower size under varying conditions attending development are a matter of common knowledge. It need only be mentioned that it is commonly recognized in horticultural practise that certain conditions of soil and treatment are conducive to the development of flowers of maximum size, and these conditions are often stated very definitely for particular plants. These facts have not escaped the attention of biometricians who have, however, for the most part concerned themselves with the discrete variations of composite flowers. Other types of flowers have, however, been considered, as for example *Ficaria ranunculoides* Moench. For this species MacLeod (1899) determined the number of stamens and the number of pistils in each of a series of flowers borne by certain plants at the beginning of the flowering season, and again in a series of flowers at the end of the season. The observations were made on the same plants (or their asexual offspring) during two successive seasons and similar results were obtained on each occasion. Although these studies were primarily concerned with correlation phenomena, the data show the same sort of differences which we have pointed out for different characters in Nicotiana. For flowers measured early in the season as reported by from these data by Weldon (1901) the mean number of stamens was 26.73 ± .15 as contrasted with a mean number late in the season of 17.86 ± .12, a difference of 8.87 ± .19, which is certainly a significant difference. Likewise an early mean number of pistils of 17.45 ± .16 decreases to 12.15 ± .12, a difference in this case of 5.30 ± .20. If we, likewise, calculate the coefficient of variability for these series we find that it increases in the case of number of stamens from 14.01 ± .41 early in the season to 18.47 ± .46 late in the season, an increase of 4.46 ± .62 per cent. Similarly for number of pistils the increase is from 22.32 ± .65 per cent to 27.88 ± .69 per cent, an increase of 5.56 ± .95. Miss Alice Lee (1902) in connection with a study of data of several series of plants of *Ficaria verna* Huds. states that "these changes while demonstrating the fact that the four series are not random samples of the same population made a the same time, are not by any means greater than the same plant in the same locality at different periods of its season or the same plant in different districts at the same period has been known to give. They are well within the limits of local environmental or seasonal changes." Although this is not quite the same character of change as the one with which we are dealing, it nevertheless seems desirable to point out the fact that wide variations in floral organs are known which can have no important genetic significance.

Our results then go to show that under the stress of external and more or less artificial conditions attending development, flower size in Nicotiana is subject to distinct and significant variations. Our principal interest in this question of the stability of the character complex, corolla size, has been centered on the effects produced by the internal, inherent, and normal ontogenetic changes that make up practically all of what we see in the development of a plant.

A varying period of vegetative development, more or less constant for a given species, precedes the appearance of the floral organs. One seems justified in assuming, at least, that the products of metabolic activity are devoted during the earliest period of development to the establishment of an adequate system of food supply at the expense of an early provision for seed production. As the plant grows older, however, the products of metabolic activity begin to be shared by both vegetative and floral organs until finally it is possible to find the vegetative characters suffering in favor of the reproductive characters. Reactions normally proceeding in one direction are forcibly reversed or perhaps reaction products are not allowed to accumulate in the usual regions and the metabolic reactions reach a point of equilibrium at a later period and in another portion of the plant. Similarly what is recognized as a periodicity in the production of flowers means the concentration of the products of metabolic activity in different regions at different times to accomplish special and individual results. That some such process must be operative is seen in the common practice on the one hand of increasing vegetative growth by the elimination of the floral organs and of restricting maximum vegetative growth to favor the early development of characters connected with the reproductive organs (cf. Cook, 1914) on the other hand. We feel that the observations tabulated on the preceding pages demonstrate the fact that under such normal, inherent conditions attending development, the size of flowers will vary to a significant extent. The first flowers produced by the vigorously growing plant are larger than those produced near the end of the flowering season when vegetative growth is at an end. An elimination of all the seed that starts to mature is effective in shifting the whole normal period of development. When the formation of seed is prevented, the normal life of the plant may be doubled and the size of the flowers it bears will remain remarkably constant throughout the entire duration of life.

In the light of these facts, the behavior of flower size in the case of the almost completely sterile F_1 hybrids dealt with in the preceding pages, is interesting and significant. These species hybrids are in general replicas on a larger scale of their *N. Tabacum* parent, but they inherit a short lived perennial habit from their *N. sylvestris* parent. Under field conditions as they exist here these F_1 hybrids reach their maximum vegetative and floral development relatively early in the growing season normal for their parents, and maintain it with only slightly diminished vigor for four or five months. There follows a rest period of from one to three months during which the plants are only feebly flowering and portions of the vegetative organs die back. With the rainy season there comes the production of new laterals and new flowers which are larger than the flowers produced toward the end of the previous period of flowering and of practically the same size as those borne at the beginning of the previous season. In other words, the flower size of plants the second year on their own roots, at the beginning of their flowering season, is the same as that of the early flowers produced the first year. This same fact is true of the succeeding year; *i. e.*, the third season of flowering. The rest period was, of course, artificially accomplished by cutting back the plants when they began to go out of flower and so show signs of dying back. These hybrids which produce no self-fertilized seed and almost no open pollinated seed show a striking diminution in size of flowers as the flowering season advances, and after a period of rest begin again to produce large flowers. As the second flowering season advances the size of the flowers again grows less. The significance of these facts in another connection has been commented upon elsewhere (Goodspeed, 1913, 1).

With reference again to the relation of the inherent and normal conditions attending development to the size of the flower, we have seen that the flower itself has a period of increase in size after opening which is sufficiently great to be of importance. A haphazard selection of flowers to be measured might give a great enough preference to flowers not fully developed to modify the significance of the mean flower size determined. The same is true for flowers produced in different parts of the plant. It has been shown that the flowers produced on an inflorescence going out of flower or covered with developing seed capsules are smaller than those on a terminal or lateral inflorescence just coming into flower.

Throughout the discussion of the tables given above, attention has been called to the difference between the behavior of corolla spread and corolla length under the influence of various conditions. This difference is sufficiently marked to leave little room for doubt that the conditions attending development do not tend to accomplish the same result with respect to the two types of measurement which together express the flower size in Nicotiana. The facts as noted may or may not be of any special significance as far as the general question of the degree of correlation between length and spread of corolla is concerned. As noted by East (loc. cit. p. 18) it is by no means uncommon to find an individual flower with a very broad corolla limb and comparatively short corolla tube"; "but one never finds inverse extremes in the same individual." Our results show, however, that there are a considerable number of individual flowers per plant which possess only a little less than the normal length of tube but a very small diameter of corolla limb. East further states (p. 182) with reference to the correlation between spread and length in an F_2 population, that "perfect coupling of certain factors together with independent combination of others may be possible; partial coupling arising from a peculiar gametic distribution may be equally possible." Our results appear to favor East's assumption of perfect coupling of certain factors with independent combination of others, and to indicate that the perfect coupling of factors may be simply due to the fact that these factors are common to length and spread of corolla.

The sum of the conclusions above drawn makes it seem possible to state definitely that flower size, although very evidently not so markedly modified by environmental conditions as height of plant, leaf size, etc., still is not a stable character complex and is subject to marked modifications under the stress of both internal and external conditions attending development. The demonstration of these facts makes necessary in the future a more detailed description of technique and general method of flower size investigations than is ordinarily included in the published reports. On general principles it would seem rather necessary in a report on flower size inheritance to mention something of the technique of taking the measurements and at least a statement as to the number considered necessary to determine a fair mean for the various individuals of the populations reported upon. The facts brought out in the preceding pages serve to show how great an influence the number of measurements taken, the period of measurement, the age of the plants, and the age of the flowers, etc., will have upon a flower size distribution.

V. Summary

In connection with the taking of some 25,000 measurements on the length and spread of corolla limb of flowers on plants of *N. Tabacum* var. *macrophylla* and three hybrids between *N. Tabacum* varieties and *N. sylvestris* the following facts were observed.

1. When the plants first come into flower the spread and length of corolla is greater than the spread and length of flowers produced on the same plants later in the growing and flowering season. This difference in size is more striking in the case of corolla spread than in the case of corolla length. Within a month or six weeks the average

spread of corolla may drop 6 mm. and the average length as much as 4.5 mm. In such a case for flowers on individual plants the difference will be greater still.

2. The F_1 *N. Tabacum* × *N. sylvestris* hybrids possess the short-lived perennial habit of *N. sylvestris* and will bloom a second and third season on their own roots. The first flowers produced the second season were of approximately the same size as the first flowers produced the first year and were considerably larger than the flowers produced toward the end of the first period of flowering. The difference in size was greater for spread of corolla than for length of corolla.

3. The removal of all open flowers during the normal flowering season will keep up the flower size to nearly that of the first flowers produced and in some cases will double the life of the plant. There is a distinct relation between the presence of developing seed on an inflorescence and the size of the flowers produced thereupon. This relation is analogous on the one hand to the agricultural practice of "topping" tobacco plants to favor vegetative luxuriance and on the other hand to the restricting of the vegetative growth in cotton to favor an earlier development of fruiting branches and thus an earlier crop.

4. Flowers that are apparently fully expanded but with the anthers unopened are smaller than fully opened flowers after anthesis. Similarly there is a striking difference in the size of flowers produced on a terminal inflorescence that is almost through flowering and on a lateral inflorescence that is just in full bloom. This difference holds for the semi-sterile F_1 hybrids as well as for the parent *N. Tabacum* var. *macrophylla* where many seed capsules are developing on the older inflorescences. Spread and length of corolla are equally diminished in size when flowers on older terminal inflorescences are compared with those on lateral inflorescences in full bloom. With reference to the difference in size of flowers before and after the dehiscence of the anthers, the spread of corolla is relatively more greatly increased after anthesis than the length.

5. The flowers on cuttings taken from plants in the field and grown in pots in the greenhouse are smaller than those borne on the plant from which the cuttings were taken. This is more strikingly evident in the case of spread than in the case of length of corolla.

6. Under favorable and unfavorable conditions of greenhouse culture flower size will vary distinctly and in the same direction as vegetative characters under such conditions.

7. The spread of corolla limb and the length of corolla tube do not behave in the same manner with reference to the operation of the various factors which are shown to be of importance in determining flower size in Nicotiana. Length of corolla is more stable than spread of corolla, which is rather readily subject to modification under the stress of internal and external conditions attending development.

8. The only true distribution representing the flower size of a population must be based upon measurements which, for each plant, extend over the greater part of the period of flowering normal for the given species or hybrid group or cover an identical portion of the flowering period of each plant. If in a large F_2 hybrid population, some plants are measured early in the flowering season, others in the middle of the flowering season, and the remainder late in the flowering season an unusually extensive range of variation in the distribution resulting may be due to modifications in flowering size dependent upon inherent ontogenetic processes and genetically of little significance.

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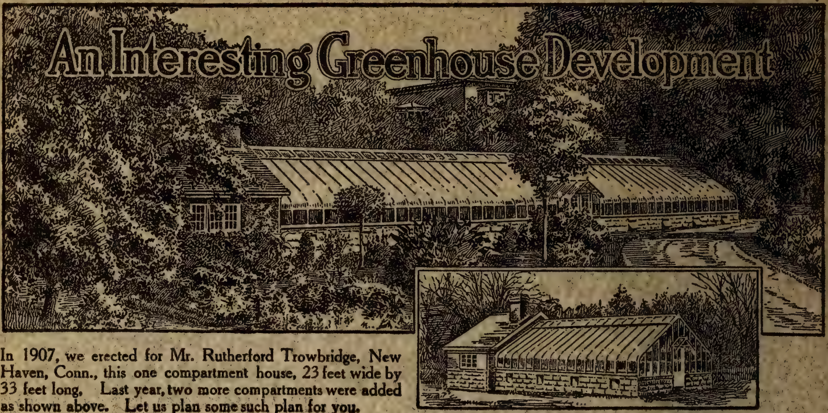

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THE UTILIZATION OF CERTAIN PENTOSES AND COMPOUNDS OF PENTOSES BY GLOMERELLA CINGULATA*

Lon A. Hawkins

Compounds of the pentose sugars are generally present in the cell walls of plants. These compounds apparently may be composed of pentoses alone or in combination with sugars of other groups. The compounds of pentoses alone are frequently called pentosans. When they are considered to be derived from a single pentose, they may be referred to more specifically, as, for example, araban and xylan, which are considered to be the polysaccharides of arabinose and xylose respectively. The widespread occurrence of these compounds in plants has led to numerous investigations as to their utilization by organisms. The question as to whether or not animals and plants secrete enzymes capable of splitting the complex pentosans to their constituent sugars has also received some attention. A discussion of the results of some of these investigations will follow. For a more comprehensive bibliography the work of Swartz[1] may be consulted.

It has been shown by Lindsey and Holland,[2] Slowtzoff,[3] Goetze and Pfeiffer,[4] Swartz,[5] and others that pentosans as such disappear in

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* Published by permission of the Secretary of Agriculture.

[1] Swartz, Mary Davies. Nutrition Investigations on the Carbohydrates of Lichens, Algae, and related substances. Trans. Conn. Acad. Arts and Sciences **16**: 247–382. 1911.

[2] Lindsey, J. B., and Holland, E. B. Concerning the Digestibility of the Pentosans. Ann. Rep. of Mass. Agr. Exp. Sta. 1894: 175–188.

[3] Slowtzoff, B. Ueber das Verhalten des Xylans im Thierkörper. Zeitschr. Physiol. Chemie **34**: 181–193. 1901–2.

[4] Goetze, K., and Pfeiffer, Th. Beiträge zur Frage über die Bildung resp. das Verhalten der Pentaglykosen im Pflanzen- und Tierkörper. Landw. Versuchs-Stationen **47**: 59–93. 1896.

[5] Swartz, Mary Davies. *Loc. cit.*

[The **Journal** for July (**2**: 311–374) was issued 18 Aug 1915]

passing through the alimentary canal of higher animals, but it seems doubtful from the work of Seillière[6] and of Swartz that the digesting enzymes are secreted by the higher animals themselves. Seillière attempted to show that the digestion of xylan by various animals was not carried out by the intestinal or pancreatic juices of these animals but by the bacteria present in the intestines. His work seems to be corroborated by the investigations of Swartz. Seillière[7] showed further, however, that the extracts of the digestive organs of snails hydrolyzed xylan, and later obtained similar results with other molluscs[8] and also with the larvae of certain Coleoptera.[9]

The question of the utilization of pentoses and pentosans by plants, especially by fungi, has received some attention. Behrens[10] has shown that *Botrytis vulgaris* and some Penicilliums can utilize both arabinose and xylose as a source of carbon. Went[11] obtained good results when growing *Monilia sitophila* (Mont.) Sacc. upon xylose. Cross, Bevan and Smith,[12] Schöne and Tollens,[13] and Cross and Tollens[14] consider that the disappearance of pentoses in fermenting mixtures of sugars is due to the utilization of pentoses in the building up of the yeast plant. Krüger[15] has recently shown that a species of Gloeosporium parasitic upon apple, which he calls *Gloeosporium fructigenum germanicum*, can utilize arabinose. Other writers have shown that the pentoses can be used by fungi, but it is generally agreed that they can not be fermented.

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[6] Seillière, G. Sur la digestion de la xylane chez quelques mammifères herbivores. Compt. Rend. Soc. Biol. **64**: 941–943. 1908; Sur la digestion de la xylane chez les mammifères. Compt. Rend. Soc. Biol. **66**: 691–693. 1909.

[7] Seillière, G. Sur la présence d'une diastase hydrolysant la xylane dans le suc gastro-intestinal de L'Escargot. Compt. Rend. Soc. Biol. **58**: 409–410. 1905.

[8] Seillière, G. Sur la présence de la xylanase chez différents Mollusques gastéro podes. Compt. Rend. Soc. Biol. **59**: 20–22. 1905.

[9] Seillière, G. Sur une diastase hydrolysant la xylane dans le tube digestif de certaines larves de Coléoptères. Ibid. **58**: 940–941. 1905.

[10] Behrens, J. Beiträge zur Kenntnis der Obstfäulnis. Centralbl. Bakter. und Parasit. Abt. II **4**: 547–553. 1898.

[11] Went, F. A. F. C. *Monilia sitophila* (Mont.) Sacc., ein technischer Pilz Javas. Centralbl. Bakter. und Parasit. Abt. II **7**: 591–598. 1901.

[12] Cross, C. F., Bevan, E. J., and Smith, Claude. The Carbohydrates of Barley Straw. Journ. Chem. Soc. **73**: 459–463. 1898.

[13] Schöne, A., und Tollens, B. Ueber die Gärung der Pentosen. Journ. Landw. **49**: 29–40. 1901.

[14] Cross, W. E., und Tollens, B. Versuche über das Verhalten der Pentosen in gärenden Mischungen. Journ. Landw. **59**: 419–428. 1911.

[15] Krüger, Frederich. Beiträge zur Kenntniss einiger Gloeosporien I und II. Arbeit. Kaiserl. Biol. Anstalt Land und Forstwirt. **9**: 233–323. 1913.

In regard to the utilization of pentosans by fungi it seems probable from the work of Czapek[16] and Schörstein[17] that certain components of the cell walls of woody tissues are rendered soluble and utilized by some fungi. The last mentioned writer reaches the conclusion that xylan is digested by *Merulius lachrymans*. He bases his conclusion upon the observed difference in optical rotation of extracts of wood before and after it had been acted upon by the fungus. Swartz[18] found apparently that the pentosans of *Rhodymenia palmata*, a marine alga, were hydrolyzed to a reducing substance by "Taka diastase." Duggar and Davis[19] recently announced that they were unable to demonstrate the presence of a pentosanase in Fucus.

It seems then that pentosans and pentoses are of some value as food for higher animals but that these organisms probably secrete no enzymes capable of hydrolyzing pentosans. Some of the invertebrates can utilize pentoses readily and seem to be able to hydrolyze some pentosans. Pentoses have been found to be a good source of carbon for certain fungi and there is evidence that some pentosans may be broken down through the action of enzymes secreted by fungi. The products of such enzyme action have apparently not been identified.

It was to obtain more information upon the effect of parasitic fungi on pentoses and their compounds that the experiments described in this paper were planned and carried out. The study includes a series of experiments on the effect of *Glomerella cingulata* (Stonem.) S. & v. S. upon pentose compounds in the apple fruit, a series on the utilization of pentoses and certain of their compounds as sources of carbon for this fungus, and experiments upon the effect of the extract of the fungus mycelium under aseptic conditions on xylan.

The fungus was isolated from apples kindly furnished by Dr. C. L. Shear and was maintained in stock culture in tubes of cornmeal agar throughout the study. In the experiments on the effect of the fungus upon the pentose-containing compounds in apples practically the same methods of sampling and analysis were used as in a former study of peaches.[20] The different compounds were determined in the two halves of the same fruit, one portion of which had been inoculated with the fungus while the other was retained sterile as a control. In the determination of the furfurol-yielding material it was found that the percentage of this substance in the rotten half was considerably less than in the sound portion. The fungus apparently used the furfurol-yielding constituents of the apple fruit. Several apples were then prepared and inoculated. After two weeks the sound and rotten halves were sliced up and extracted with alcohol. The furfurol-yielding material was then determined in the extract and solid portions separately. The results are given in Table I. All data were calculated as percentage of the original wet weight of the portion of the apple used.

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[16] Czapek, F. Zur Biologie der holzbewohenden Pilze. Bericht. Deutsch. Bot. Ges. 17: 166–170. 1899.

[17] Schörstein, J. Zur Biochemie der Holzpilze. Centralbl. Bakter. und Parasit. Abt. II 9: 446–447. 1902.

[18] Swartz, Mary Davies. *Loc. cit.*

[19] Duggar, B. M., and Davis, A. R. Enzyme Action in *Fucus vesiculosus* L. Ann. Mo. Bot. Gard. 1: 419–426. 1914.

TABLE I

Percentage of Alcohol-soluble, Alcohol-insoluble, and Total Furfurol-yielding Material in Sound and Rotten Halves of Apple, Each Substance Determined in Sound and Rotten Halves of Same Fruit

Alcohol-soluble		Alcohol-insoluble		Total	
Sound half	Rotten half	Sound half	Rotten half	Sound half	Rotten half
0.12	0.18	0.62	0.49	0.74	0.67
0.12	0.16	0.71	0.50	0.83	0.66
0.14	0.27	1.11	0.93	1.25	1.20

From the data in Table I it is apparent that the total percentage of furfurol-yielding material and the percentage of alcohol-insoluble furfurol-yielding material were higher in the sound portion than in the corresponding rotten half of the apple, but that the percentage of alcohol-soluble furfurol-yielding material was higher in the rotten half.

Pentose sugars are readily soluble in 80 percent alcohol while most of the furfurol-yielding material in the sound apple is not. The increase in alcohol-soluble furfurol-yielding material during the early stages of the rot therefore indicates that some compounds containing pentoses were broken down. That this was due to the action of the fungus and not to the autolysis of the dead apple tissue was evident from the fact that when portions of apple in which the cells had been killed with chloroform were allowed to stand under aseptic conditions for three weeks they contained practically the same percentage of both alcohol-soluble and alcohol-insoluble pentosans as control portions of the same apples in which the cells had not been killed.

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[20] Hawkins, Lon A. Some Effects of the Brown Rot Fungus upon the Composition of the Peach. Amer. Journ. Bot. **2**: 71–81. 1915.

When the fungus was allowed to act upon the apple four weeks or more the percentages of furfurol-yielding material in the alcohol extract and in the solid residue were both higher in the sound half. It is evident then that the fungus is able to break down the pentose-containing compounds, whether they be pentosans or compounds of the pentoses with other sugars, and to use the pentoses. There is no evidence at hand, however, to show which pentoses can be used or what compounds of the pentoses are most readily broken down by the fungus. This was made the subject of further experimentation. An attempt was made to determine the value of xylose, arabinose, xylan and arabin as compared with glucose as sources of carbon for this fungus.

The fungus was grown on a solution of nutrient salts to which the sugars and compounds of the pentoses were added. The medium was prepared according to a formula used by Hasselbring[22] in his work on Penicillium. It was composed of NH_4NO_3 1 g., KH_2PO_4 0.5 g., $MgSO_4$ 0.25 g., and 100 cc. H_2O. To this was added the carbohydrate usually in sufficient quantity to make a 1 percent concentration. Three hundred cc. Erlenmeyer flasks were used for the cultures. In each of these 100 cc. of the culture solution was placed. These solutions were inoculated with the fungus by transferring conidiospores from the stock cultures with a sterile needle. After a suitable period the felt of mycelium was removed from each flask separately, washed with a little water, placed in a tared, glass-stoppered weighing bottle and covered with alcohol. The alcohol was driven off at low temperature, about 60° C., and the weighing bottles with mycelium dried to constant weight in a vacuum drying oven at 78° C.

The sugars, the arabin, and a small part of the xylan used in these experiments were purchased from a chemical supply house. The rest of the xylan was prepared from rye straw, most of it according to a method similar to that used by Schöne and Tollens[23] for the preparation of xylan from jute. The ground straw was extracted with 2 percent solution of ammonia and then digested 48 hours with a 6 percent solution of KOH, 6 liters to each original half kilogram of straw. The extract was pressed out, filtered through cloth and the xylan precipitated by adding an equal volume of 95 percent alcohol to the solution. It was then filtered and the precipitate neutralized with a solution of HCl in alcohol. The xylan was washed in a Buchner funnel with 60 percent alcohol until the washings gave no test for chloride with silver nitrate. It was then dried with absolute alcohol and ether, ground up in a mortar, passed through a fine sieve and was ready for use. In order to prove that the preparation was a compound of xylose sugar, a quantity of the xylan was hydrolyzed with sulphuric acid according to the method of Wheeler and Tollens.[24]

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[22] Hasselbring, H. The Carbon Assimilation of Pencillium. Bot. Gaz. **45**: 176–193. 1908.

[23] Schöne, A., und Tollens, B. Untersuchungen über die Pentosane der Jute, der Luffa, und der Biertreber. Journ. Landw. **49**: 21–28. 1901.

The mixture of xylan and 5 percent sulphuric acid was heated on the steam bath for six hours. The acid was then neutralized with magnesium-free calcium carbonate, filtered, and the filtrate evaporated nearly to dryness under reduced pressure. The residue was taken up with hot alcohol and treated with animal charcoal to decolorize. The solution was concentrated in a vacuum desiccator over sulphuric acid and in a few days the sugar crystallized out. It was recrystallized from alcohol several times. The crystals were white and had the characteristic form of xylose. They melted at 143°–144° C., uncorrected. The sugar had a specific rotation, $[a]_D$, of + 18.92 as compared with + 19.22[25] for xylose. It reduced Fehling's solution, formed furfurol which was precipitated as the phloroglucid when boiled with a hydrochloric acid solution of phloroglucin. The osazone was prepared in the usual way and crystallized as bright yellow needle-like crystals, insoluble in cold water, soluble in hot, and melting at 160° C,, uncorrected, as compared with 160° C. for xylosazone according to Tollens.[26] The sugar was apparently xylose.

In the preparation of xylan, Salkowski's method[27] was also used.

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[24] Wheeler, H. J., und Tollens, B. Ueber die Xylose oder den Holzzucker, eine zweite Penta-glycose. Zeitschr. Vereins Rübenzucker-Industrie Deutsch. Reichs **39**: 848–868. 1889.

[25] Parcus, E., und Tollens, B. Ueber die Mehr- oder Weniger-Drehung (Multi-Rotation oder sog. Birotation und Halbrotation) der Zuckerarten. Liebig Ann. **257**: 160–178. 1890.

[26] Tollens, B., et al. Untersuchungen über Kohlenhydrate. Zeitschr. Vereins Rübenzucker-Industrie Deutsch. Reichs **41**: 885–911. 1891.

[27] Salkowski, E. Über die Darstellung des Xylan. Zeitschr. Physiol. Chem. **34**: 162–180. 1901–02.

According to this method the ground straw was boiled in 5 percent KOH for 1 hour, the extract pressed out with a fruit press and filtered through cloth. Fehling's solution was added in the proportion of one half liter to every 300 g. of straw used. The precipitate was separated by filtration, washed and treated with an alcoholic solution of hydrochloric acid. It was washed several times with 60 percent alcohol, dried and dissolved in dilute KOH and again precipitated with Fehling's solution. It was then washed and acidified as before and finally freed from chloride and dried.

Salkowski considered that xylan prepared according to this method was practically free from araban and other hemicelluloses, cellulose and starch, and that it was nearly pure.

This preparation yielded 74.0 percent xylan, according to Krober's tables,[28] when analyzed according to Tollens' phloroglucid method for the determination of pentosans, which is one percent more than was found in the xylan prepared according to Schöne and Tollens' method and analyzed in the same way. The commercial preparation of xylan used in these experiments gave 86.1 percent of the theoretical amount. The xylan prepared according to Salkowski's method dissolved in 1 percent KOH had a specific rotation, $[a]_D$, of $-83°$. It contained 0.5 percent ash. Swartz[29] mentions some which she prepared which yielded 72.0 percent of the theoretical amount according to Krober's tables. She found the specific rotation of one sample to be $-83°$. Tollens gives the specific rotation of xylan from wheat straw as $-84.1°$. Swartz's determinations of ash in the xylan were somewhat higher than those obtained in the present study while Salkowski reports that some of his preparations contained as low as 0.7 percent ash.

The xylans prepared by both Tollens's and Salkowski's methods were tested for galactan and methyl pentosans with negative results. No starch was present. It is apparent from the above described experiments that the compounds prepared were composed largely of xylose.

The first series of experiments was made with the two pentose sugars as compared with glucose as a source of carbon. The sugars were added to the solutions of nutrient salts in quantity to make one percent concentration. In preparing the culture media the solutions of salts were made up at a somewhat higher concentration than finally desired and sterilized in the autoclave, then diluted to the proper concentration by the addition of the sugar solutions which had been sterilized separately in a steamer. The culture solutions were inoculated and the fungus allowed to grow twelve days in an incubator at about 28° C. At the end of this time the mycelium was removed and dried to constant weight. The culture solutions were tested for reducing sugars, and the solutions which originally contained xylose or arabinose were tested for pentoses. Traces only were found. The sugar had been almost entirely removed from the solution by the fungus. The weight of the mycelium from the twelve flasks is shown in Table II.

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[28] Wiley, H. W., et al. Official and Provisional Methods of Analysis. Association of Official Agricultural Chemists. U. S. Dept. Agr., Bur. Chem. Bull. 107. 1907.

[29] Swartz, Mary Davies. *Loc. cit.*

TABLE II

Comparative Yields of Mycelium, Expressed in mg. Dry Weight, from Cultures of Glomerella cingulata on Nutrient Salt Solutions with Glucose, Arabinose or Xylose as Source of Carbon

Glucose	Arabinose	Xylose
365.1	382.0	401.4
347.5	372.3	383.8
317.3	337.2	380.1
365.3	349.5	386.1

The pentoses seem to be slightly better sources of carbon than glucose. Xylose is apparently the best, though the difference is not great. It is very evident that the pentoses are readily utilized by this fungus.

A series of culture solutions was next made using arabin and two xylans, one prepared from rye straw and the other the commercial preparation. The yield in dry weight of fungus mycelium with the amount of furfurol-yielding material used in each case is given in Table III. The amount of arabin or xylan used was determined by

TABLE III

Comparative Yields of Mycelium and Amount of Pentosan Used with Cultures of Glomerella cingulata Grown upon Nutrient Salt Solutions with Xylan or Arabin as the Source of Carbon

Commercial Xylan		Xylan Prepared from Rye Straw		Arabin	
Yield, mg.	Percentage of Xylan Used	Yield, mg.	Percentage of Xylan Used	Yield, mg.	Percentage of Arabin Used
202.2	86.0	230.9	88.0	74.3	15.0
196.0	85.0	....	...	64.6	14.0
179.9	90.0	....	...	98.2	13.0
205.4	87.0	....	...	79.8	15.0

difference between the amount remaining in the culture solutions and the amount recovered from uninoculated control flasks, prepared in exactly the same way as the flasks for cultures. The flasks were kept in an incubator at 28° C. throughout the course of the experiment, which lasted fifteen days.

It is obvious from Table III that these compounds of pentose sugars can be utilized by the fungus as sources of carbon. It is apparent that the yield of mycelium was less after fifteen days with either arabin or one of the xylans as the source of carbon than in the case of any one of the three sugars in twelve days. A much larger amount of the compound remained in the solution than in the case of the sugars which were removed almost quantitatively. It is evident then that xylan and arabin are not as readily available for the fungus as the pentose sugars themselves.

A comparison of xylan and arabin shows that the yield of dried mycelium was considerably more than twice as great from the cultures containing xylan, while a much higher percentage of the material was removed from the solution. The conclusion is obvious then from the table that xylan, although not as good a source of carbon as xylose, arabinose, or glucose is readily available and can be utilized by the fungus. It also seems probable that in the utilization of the xylan it is broken down into simpler compounds and that one or several steps in its digestion and assimilation is the hydrolysis of xylan to xylose, the sugar. If this is the case there is probably an enzyme secreted by the fungus which brings about this hydrolysis.

A series of experiments was planned and carried out to see if the extract of the fungus was able to hydrolyze xylan to xylose under aseptic conditions. The fungus was grown on the mixture of nutrient salts already described with gum arabic or glucose as a source of carbon. The cultures were allowed to grow about three weeks before the mat of mycelium was removed. It was separated from the culture solution, washed with a little water and ground up in a mortar. The resulting pulp was then placed in a flask with water and a little chloroform and allowed to stand with frequent shaking for 24 hours. It was then filtered and the extract used in the digestion experiments. The xylan was prepared by weighing small quantities, 0.2 or 0.3 g., into 100 cc. flasks to which 25 cc. of water was added. The mixture was then boiled, cooled, and 25 cc. of the extract from the fungus mycelium was added to each flask. The mixtures were neutral to litmus. Several of the flasks containing the xylan and extract of the fungus were again boiled as controls. Chloroform was finally added to all the preparations as an antiseptic. The flasks were then placed in an incubator at a temperature of approximately 30° C. and allowed to remain with frequent shaking for the required time. To see whether the preparations were contaminated with microorganisms frequent inoculations were made from the mixtures to various culture media. No organisms were found.

The first series of experiments was carried out with six preparations for each experiment, all alike excepting that three had been boiled. After these had remained in the constant temperature chamber from five to eight days they were removed, and the contents of the flasks washed into beakers with 95 percent alcohol. Sufficient alcohol was added to bring the mixture up to about 80 percent alcohol, thus precipitating the unchanged xylan. The mixture was filtered and the alcohol evaporated from the filtrate on the steam bath. One pair of preparations consisting of one unboiled and one boiled control was used for the determination of the furfurol-yielding substance. A similar pair was used for the determination of the reducing substance. These last were cleared with neutral lead acetate, filtered, the excess lead precipitated as oxalate, and the mixture filtered again. The sugar was determined in the filtrate, using Allihn's modification of Fehling's solution.[30] The dry cuprous oxide was weighed directly. The third pair of preparations was usually used for the preparation of the phenylhydrazine derivative which will be taken up later. It has been shown earlier in this paper that xylose is soluble in 80 percent alcohol, while xylan is not; also xylose is a reducing sugar, forms furfurol when boiled with HCl and reacts with phenylhydrazine to form a characteristic osazone. It is apparent then that if the amount of alcohol-soluble furfurol-yielding material and reducing substance is greater in the unboiled preparation it will be evidence that a pentose sugar results from the action of the extract of fungus mycelium upon the xylan. This evidence will be strengthened if the phenylhydrazine derivative is similar to xylosazone. The results of this series of experiments are shown in Table IV.

The phenylhydrazine derivative was prepared in the usual manner in the third unboiled preparation in all the experiments given in Table IV. In all cases it proved to be the same, bright yellow needle-

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[30] Wiley, H. W., et al. *Loc. cit.*

TABLE IV

Comparative Effect of Boiled and Unboiled Extract of Fungus Mycelium upon Xylan from Rye Straw as Shown by Alcohol-soluble Furfurol-yielding Material and Substance Reducing Fehling's Solution

Duration of Experiment	Amount of Xylan in Each Preparation	Cuprous Oxide Derived from Material Reducing Fehling's Solution.		Amount of Pentoses	
		Unboiled	Boiled	Unboiled	Boiled
	g.	mg.	mg.	mg.	mg.
6 days	0.3	123.8	31.5	67.5	9.2
8 "	0.3	93.6	18.1	52.1	8.3
8 "	0.2	70.5	12.1	46.7	8.1
10 "	0.3	133.0	31.4	67.8	9.5
10 "	0.2	108.2	17.6	47.4	9.5

shaped crystals soluble in hot water, insoluble in cold, and melting at 161°–162° C. uncorrected, as compared with 161° C. for xylosazones prepared from the xylose sugar obtained from rye straw and from the commercial xylose as used in the experiments already described. Some of the control preparations were treated in a like manner, but the quantity of osazone formed was so small that it was impossible to identify it with certainty.

It is obvious from Table IV that there is much more alcohol-soluble substance which reduces Fehling's solution and furfurol-yielding substances in the unboiled preparations than in the boiled controls. That this increase is not due to the autolysis of the filtered extract of the fungus mycelium itself was proved by the fact that when the extract was accorded exactly the same treatment as the unboiled preparations no appreciable amount of reducing substance was found and no measurable amount of furfurol-yielding material. The above mentioned considerations and the fact that the phenylhydrazine derivative is similar to the osazone of xylose seems to show that xylose results from this action of the unboiled extract of fungus mycelium upon the xylan.

Whether this hydrolysis takes place immediately or the amount of alcohol-soluble reducing substance and furfurol-yielding material present at the completion of the experiment is the result of a gradual breaking down of the xylan is now shown by the results already given. Further experimentation was necessary to obtain evidence upon this point. In these experiments twelve or more similar preparations were made, as already described, several of which were boiled as before for controls. The flasks were placed in an incubator and shaken frequently. At intervals some of the preparations, both unboiled and boiled, were removed from the incubator, the xylan precipitated and alcohol-soluble furfurol-yielding substances determined.

In the experiments, the results of which are given in Table V, both the reducing substance and the furfurol-yielding material are given. In the other experiments (Table VI) only the latter was determined.

Table V

Production of Alcohol-soluble, Furfurol-yielding Material, and Substance Reducing Fehling's Solution, by Action of Extract of Fungus Mycelium upon Xylan from Rye Straw. 0.3 *g. Xylan in Each Preparation. Yield in mg. Pentoses and mg. Cuprous Oxide*

Duration of Action	Unboiled Preparations		Boiled Preparations	
	Cuprous Oxide	Pentoses	Cuprous Oxide	Pentoses
	mg.	mg.	mg.	mg.
Beginning......	......	.....	23.7	9.5
4 days........	71.4	57.2	.....	.....
8 "	99.8	63.5	19.4	9.0
12 "	100.4	66.5	.....	.....
16 "	100.8	66.3	20.6	9.4

It is evident from Tables V and VI that the hydrolysis of the

Table VI

The Production of Alcohol-soluble, Furfurol-yielding Substances by Action of Extract of Fungus Mycelium upon Xylan. Yield as mg. Pentoses

No. of Experiment	Amount of Xylan in Each Preparation in g.	Duration of Experiment	Unboiled		Boiled Control
			1	2	
			mg.	mg.	mg.
1	0.3	At beginning	.....	.....	9.7
		20 hours	34.1	35.0	.....
		44 "	40.0	38.2	10.5
		68 "	41.2	43.3	.....
		106 "	47.3	44.0	8.8
2	0.3	At beginning	.....	.....	9.9
		20 hours	19.0	19.0	.....
		42 "	25.1	25.4	.....
		66 "	31.0	31.1	.....
		80 "	38.7	34.3	8.1
3	0.2	At beginning	.....	.....	8.8
		12 hours	13.5	13.8	.....
		36 "	15.9	16.1	.....
		70 "	17.2	17.1	.....
		94 "	17.8	17.7	8.8

xylan did not take place immediately but that the amount of alcohol-soluble furfurol-yielding substance (xylose) increased the longer the extract was allowed to act. The rate of hydrolysis was much more rapid at first and gradually decreased almost, if not quite, to zero. In contrast to the unboiled preparation there seemed to be no increase in the alcohol-soluble furfurol-yielding material in the boiled controls; that is, heating to 100° C. apparently rendered the extract incapable of affecting the xylan.

While it seemed probable that xylose was liberated by the action of the extract of the fungus upon xylan it was deemed advisable to attempt to crystallize the sugar from the alcohol-soluble portion of the preparation. In order to obtain a sufficient quantity, a number of flasks of the xylan and extract of the fungus mycelium were prepared in the usual manner excepting that larger quantities were used.

These preparations were kept in the incubator for about a week, the unchanged xylan precipitated as before, the alcohol extract concentrated under reduced pressure, decolorized with animal charcoal and filtered. The filtrate was allowed to evaporate slowly over sulphuric acid in a desiccator and crystals were formed. These crystals were separated from the mother liquor and recrystallized. The substance did not melt sharply but apparently fused between 141° and 144° C. This was probably due to impurities present. The crystals reduced Fehling's solution and formed the characteristic phloroglucid when boiled with a hydrochloric acid solution of phloroglucin. The phenylhydrazine derivative was similar to the osazone of xylose as prepared in this study in color, crystal form, solubility and melting point. The compound when dissolved in water and treated with cadmium carbonate and bromine formed the characteristic crystals of the double salt of cadmium xylonate and cadmium bromide described by Widstoe and Tollens[31] and observed in the present study with xylose. These properties all agree closely with the properties of xylose.

It is evident then that xylan is hydrolyzed under aseptic conditions by the extract of the fungus and that xylose is formed. No attempt was made to determine whether intermediate products of hydrolysis were present as might well be the case.

In these experiments on the effect of the fungus *Glomerella cingulata* upon pentoses and compounds of pentose sugars it has been shown that the amount of furfurol-yielding material in the apple is decreased when the apple is rotted by this fungus. This decrease is brought about by the action of the fungus on the compounds in the apple which contain pentoses. These compounds are broken down and the furfurol-yielding material at least is used by the fungus. In this process the alcohol-soluble furfurol-yielding material is increased, which would seem to indicate that the pentose sugars are split off from the compounds in which they exist in the fruit.

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[31] Widstoe, J. A., und Tollens, B. Ueber Arabinose, Xylose und Fucose aus Traganth. Bericht. Deutsch. Chem. Ges. **33**: 132–143. 1900.

The fungus is able to utilize either glucose, xylose, arabinose, arabin or xylan as a sole source of carbon. The three sugars are most efficiently utilized, xylose perhaps the best. The fungus grows better on xylan than on arabin.

The filtered extract of the fungus mycelium is able to act on xylan under aseptic conditions with the formation of alcohol-soluble substance which reduces Fehling's solution, forms furfurol when boiled with HCl and possesses other properties of xylose. This ability of the extract is lost on boiling. The breaking down of the xylan takes place gradually and the alcohol-soluble furfurol-yielding material is found to increase the longer the extract acts upon the xylan, the rate being much more rapid, however, during the early part of the action.

A crystalline compound was obtained from the alcohol-soluble portion of the preparation of xylan which had been acted on by the extract of the fungus mycelium. This compound was apparently xylose. It is evident then that there is a xylanase present in the extract of fungus mycelium which hydrolyzes xylan to xylose.

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THE QUESTION OF THE TOXICITY OF DISTILLED WATER *

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INTRODUCTION

Attention has recently been drawn to the necessity of a physiologically balanced salt solution for water cultures. This necessity arises from the fact that in order to know the effect on organisms of certain single salts or mixtures of salts in solution, one must use a control or check solution, called by True,[1] a normal physiological solution. This solution or medium should cause no disturbance of the regular functions of the seedling. Such a culture would afford an ideal standard for comparison and one much to be desired. The usual method, however, has been to use distilled water. That such cultures for biological studies are unsuitable was shown some time ago.

In the first place, 'distilled water' is an indefinite term. As soon as the seedling roots are introduced the conductivity of the water rises rapidly, showing that salts are leached from the roots and the water remains no longer pure. Secondly, the extraction of salts, it is affirmed, suggests the means by which pure samples of distilled water exert their harmful effects. Some authors affirm a starvation theory; others, like Loeb,[2] the loss of certain necessary ion-proteid compounds; and still others, as True,[3] that certain necessary constituents, partly inorganic, are dissociated from their proper attachments in the complicated chemical and physical mechanism of the living cell. Thirdly, the harmfulness of distilled water is attributed by others to the presence in it of toxic substance derived from the apparatus or because of the method of preparation.

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* Received for publication April 10.

[1] True, R. H. Distilled Water in the Laboratory. Science n. ser. **39**: 296. 1914.

[2] Loeb, J. On Ion-proteid Compounds and Their Rôle in the Mechanics of Life Phenomena. I. The Poisonous Character of a Pure NaCl Solution. Amer. Journ. Physiol. **3**: 327. 1899–1900.

[3] True, R. H. The Harmful Action of Distilled Water. Amer. Journ. Bot. **1**: 270. 1914.

The two views, namely, that the distilled water is toxic *per se*, or that it is toxic because of the presence of poisonous substances in it, have been the principal ones held to the almost complete exclusion of any other. In this paper the writer wishes to draw attention to two other aspects of the distilled water problem. It seems that the problem is more a dynamic one than otherwise and in all probability more than one factor enters into the explanation of why seedlings cannot survive in distilled water. Right at this point it must be said that certain seedlings because of various physiological characteristics are less susceptible to distilled water injury than others. It is very probable that different varieties of seedlings and even different individuals of the same variety react differently to the same external conditions. From the work done in this laboratory but not reported the conclusion can be drawn that, providing one selects disease-free seed uniform in size, color, etc., one can detect as much individual variation in the behavior towards toxic substances in solution of seedlings of pure line seeds from one plant as in that of seedlings of a mixed progeny from unknown source. It follows from this that a medium which affords optimum conditions for one organism will not be the best for another. A normal physiological solution as mentioned above will perhaps afford optimum conditions for one kind of seedlings but not for another. Certain other salts or other proportions are better. In all probability there is no universal physiologically balanced solution. Loeb[4] states that salt water is a physiologically balanced salt solution for *such organisms as thrive in it* and the blood of normal, healthy subjects can also be considered a physiologically balanced solution. From our knowledge of balanced solutions it may be assumed that the rôle of the various salts is one of antagonistic action, a neutralization of the toxic effect of a single salt or of the harmful proportion of the various salts in the medium. That distilled water cultures of seedlings are not physiologically balanced solutions, can be assumed without much danger of contradiction. Wherein, then, lies the inappropriateness of distilled water for control cultures; and wherein lies the explanation of the injurious effect of such waters, and still further injury as the seedlings continue to grow in it? This preliminary paper will endeavor to show, *first*, that the adjustment of the seedlings to a distilled water medium is an important factor. It seems probable that injury is due to the greatness of the change. It will attempt to show *secondly*, that the possible injury which might arise from toxic substances excreted by the roots while the seedlings are growing in the water is another important factor to be considered.

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[4] Loeb, J. The Relative Toxicity of Distilled Water, Sugar Solutions and Solutions of Various Constituents of the Sea Water for Marine Animals. Univ. Calif. Publ. 1: 62 and 69. 1903.

In lieu of any historical resumé, mention is here made of the literature and references cited by Livingston,[5] Hoyt[6] and True.[7] These papers give a very complete summary of the history of the work done up to date by both animal and plant physiologists. It is interesting to note that the work is about evenly divided between these two divisions of biology.

Materials and Methods

The distilled water used in these experiments was of low conductivity, $2.5 - 1.4 \times 10^{-6}$. A battery of stills was set up and kept in operation all day and on the following days until the required amount of water was obtained. Then the experiment was started. The waters were stored in clean Jena bottles for no longer than the period of experimentation. Each individual still consisted of one 3-liter Jena glass flask, one condenser with spiral Jena glass inner tube, Jena glass tubing for connections where necessary and cotton plugs. The water was collected in either 5-liter, ground-glass-stoppered Jena bottles, or 3-liter ground-glass-stoppered Jena flasks.

As indicators, the roots of *Lupinus albus* were selected. The seeds were kindly given me by Dr. True. These were soaked over night in either tap water or distilled water and suspended in damp chambers so as to form straight roots. No trouble was experienced in obtaining straight roots. When of the proper length the roots were marked at a point 10 or 15 mm. behind the tip and inserted in the loop of paraffined copper hooks. These were never allowed to touch the water, and only the radicles were immersed. These hooks were then arranged around the rim of Jena beakers of 250 cc. capacity. All glassware was previously thoroughly washed, rinsed, soaked in sulphuric acid-potassium dichromate solution for a few hours, then washed, thoroughly steamed and finally rinsed with the test water before being used in the experiment. The growth rate was chosen as the criterion. The control solution was tap water. This was allowed to run freely from the faucet for at least 15 minutes so as not to use the water that had rested in the supply pipes. It was then collected in clean Jena glass flasks and put aside near a radiator to come quickly to the room temperature, the temperature at which the distilled water was maintained. The cultures were set aside on a table away from the windows and covered with paper.

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[5] Livingston, B. E. Further Studies on the Properties of Unproductive Soils. U. S. Dept. Agr., Bur. Soils Bull. **36**: 57, 71. 1907.

[6] Hoyt, W. D. Some Toxic and Antitoxic Effects in Cultures of Spirogyra. Bull. Torrey Club **40**: 333–360. 1913.

[7] True, R. H. The Harmful Action of Distilled Water. Amer. Journ. Bot. **1**: 253–273. 1914.

Preparation of Distilled Water

Only water which was prepared in contact with laboratory air of average purity is to be here considered. Absolute water prepared and used in vacuo[8]; water obtained by the combination of oxygen and hydrogen in the presence of an electric spark[9] or water distilled from a quartz distilling apparatus in contact only with air from out-of-doors dried and rid of CO_2 by the usual methods,[10] is not here used. A comparative study of all the available methods was made. Various combinations were used for the purpose of eliminating this or that source of trouble. In all twenty-six methods were tried. Livingston[11] showed that good water could be obtained by shaking distilled water with finely divided carbon black. By a process of adsorption both volatile and non-volatile substances toxic in nature are taken up. E. P. Lyon[12] obtained water of excellent quality by adding a little H_2SO_4 to the tap water in an automatic still consisting of a copper vessel and glass condenser, providing none of the condensed water touched the metal. It is suggested that the volatile toxin, ammonia, is eliminated by the H_2SO_4 treatment. Hoyt[13] made an improvement on Livingston's method by distillation with animal charcoal in the retort. A combination of the last two with an additional treatment mentioned below gave the best water in contact with air that the writer could get and one that had a conductivity of 1.4×10^{-6}.

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[8] Kohlrausch & Heydweiller. Wied. Ann. **53**: 209. 1894.

[9] Any of the good text-books on general chemistry.

[10] Method used in the research laboratory of physical chemistry, University of Illinois. Not yet reported.

[11] Livingston, B. E., Jensen, C. A., Breazeale, J. F., Pember, F. R., Skinner, J. J. Further Studies on the Properties of Unproductive Soils. U. S. Dept. Agr. Bur. Soils Bull. **36**. 1907.

[12] Lyon, E. P. Biological Examination of Distilled Water. Biol. Bull. Marine Biol. Lab. **6**: 198. 1904.

[13] Hoyt, W. D. Some Toxic and Antitoxic Effects in Cultures of Spirogyra. Bull. Torrey Club **40**: 338. 1913.

About 5 liters of tap water were first shaken up with animal charcoal and allowed to stand, with frequent shakings, for several hours, and then transferred to the retort for distillation. This length of time is hardly necessary since the adsorption equilibrium is reached in a comparatively short time. The first 500 cc. distilled over was discarded and the last 800 cc. was not distilled. The part left, the middle portion, was poured into a second retort to which a little concentrated H_2SO_4 was added and distillation started. The first 300 cc. (about) was discarded and the last 800 cc. not distilled. The middle portion proved to be a medium in which lupine seedlings grew very well and it was thought, since previous work had shown that dilute solutions of H_2SO_4 stimulated growth, that some sulphates might have passed over. Three tests were made for sulphates but none were found. It was thought that here was a suitable water, but to allay all suspicions it was again distilled in a third retort to which was added a little $Ba(OH)_2$. The middle portion from this distillation was caught in a 5-liter Jena bottle, thoroughly cleaned and steamed. The water was then aerated. The air was drawn from out-of-doors through tubes of calcium chloride, sulphuric acid and potassium hydroxide solution. This bubbling process was continued for about twenty-four hours. In this water, lupine seedlings made good growth and showed not the least sign of injury for three weeks providing the distilled water was changed at least twice daily.

In the following experiment the records were kept for a week. The experiment was continued, however, and at the end of three weeks the seedlings were in good condition but not as far advanced as the checks in tap water. There were 10 seedlings in each beaker. At the end of the first twenty-four hours the total average growth in distilled water was 33.7 mm. On the following day the average increase was 4.5 mm. The increase for each following day is 3.6 mm., 5.3 mm., 4.3 mm., 7.8 mm. and 7.3 mm. The data for tap water follow: At the end of 24 hours the total average for 10 seedlings was 34.6 mm. Increase during successive 24-hour periods: 11.7 mm., 15.2 mm., 13.5 mm., 9.6 mm., 11.6 mm., 9.1 mm. It may be noted here that the growth is a little better in tap than in distilled water, but the rate of increase is of the same order. The growth in tap water is quite luxuriant. Analysis of the tap water kindly given me by the station chemist, Prof. A. J. Patten, is here appended.

Total Solids	CaO	MgO	SO_3	Cl
Parts per 100,000	33.9	11.06	00	.025

The Problem of Adjustment

It seems that marked and sudden changes from waters of low resistance to waters of high resistance would affect the equilibrium between the physical and chemical mechanisms of the living cell. When the changes are not so great the deleterious effects will not be so great and the organism can survive in a medium, in which, if thrust suddenly, it would die. This fact also suggests another generally admitted but so often overlooked in biological studies. If comparative results are desired, the organisms used must be living under uniform conditions previous to the time of experimentation. Their future behavior depends as much on their internal nature, which is the result of their previous surroundings and past history, as on the condition of the surroundings into which they will be placed. One will not be offering a too daring interpretation if he regard this feature of adjustment as a very important one in the consideration of the injurious effect of distilled water. My experiments agree in general with the results obtained by Daniels[14] who concluded that death of Paramecia in distilled water was caused by the sudden change in media.

Peters states, "that if a change from culture liquid to pure water be made as rapidly as in these experiments (with Stentor and Paramecia) a fatal effect ensues. On account of the conception I have formed of the mode of action of the pure water, I would expect the same effect to occur even if the changes occupied a longer period of time." The inference is that the factor of adjustment is not important.

To test this theory of adjustment for lupine seedlings and distilled water, various mixtures of tap and distilled water were made. The seedlings were first placed in a beaker of tap water and allowed to grow in that for a definite time. Then all but 5 were removed and placed in a beaker containing a mixture of tap water and distilled water in the ratio of 1 : 4. After a definite time all but 5 of these were removed and placed in a mixture in the proportion of 1 : 8. This operation was repeated until 5 were placed in distilled water from the last dilution of 1 : 64. The experiment was then allowed to run 24 hours longer. Now the total growth was recorded in each beaker. At the same time that this series was set up a duplicate one was arranged and placed on the table beside the first. At the beginning of the experiment 5 seedlings were placed in each beaker. The total growth of the roots of these seedlings was recorded at the same time as the other set. In the last beaker of the first set were five seedlings that had been placed in the distilled water after they had been growing for a stated interval in 5 different dilutions of distilled and tap water. In the last beaker of the second series were five seedlings that had been immediately placed in distilled water. In this way one set of seedlings was exposed to the influence of distilled water after gradual changes and the other immediately. In another experiment the method was varied, in one way, by using mixtures containing different ratios of tap and distilled water, and in another way by arranging the experiment so that 10 seedlings could be put into the last beaker of the series. The change was first made daily, in another experiment twice a day, and in the final experiment four times a day. In one experiment the records of root growth were made at every change in every beaker. The results of these experiments show that better root growth was obtained in distilled water when changes from tap to distilled water were made gradually instead of suddenly. In the following table T and D stand respectively for tap and distilled water.

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[14] Daniels, J. F. The Adjustment of Paramecia to Distilled Water and Its Bearing on the Problem of the Necessary Inorganic Salt Content. Amer. Journ. Physiol. **23**: 48–63. 1908–9.

Where Changes are Sudden

	Tap	$\frac{1}{4}T+\frac{3}{4}D$	$\frac{1}{16}T+\frac{15}{16}D$	$\frac{1}{32}T+\frac{31}{32}D$	$\frac{1}{64}T+\frac{63}{64}D$	Dist.
Total growth in mm.	34.3	36.5	37.4	31.2	31.5	27.5
Where Changes are Gradual						
Total growth in mm.	38.3	43.4	38	36.4	36.3	34.3

Greater differences might have been obtained had a more sensitive indicator been selected. As far as our experience goes lupines are not very sensitive to distilled water injury. When exposed to sudden changes they react in a moderate way. When in distilled water frequently changed to rid the medium of the toxic substances or unbalanced condition of the organic and inorganic material therein, they live until the nutritive substances in the cotyledons have been exhausted. Frank's[15] statement that lupine seedlings are poisoned by distilled water has been questioned by Schulze[16] who states that in all probability the effect produced is the result of impurities in the water. From our experience with lupines we would prefer to agree with Schulze.

It may also be observed from a study of the experimental data above that the change from the last dilution to distilled water affects the growth rate more than changes in the other dilutions. Were the cause of injury due to osmotic effects, this would show up in the first or greater change but here we always have a better growth rate than in any of the other dilutions. That osmotic effects can be ruled out has been shown by Loeb, True and others. Can the same be said of low salt content? Is the low salt content of the medium the cause of injury? J. F. Daniels[14] showed that the destructiveness of distilled water cannot be due to its low content of inorganic salts. Two drops were diluted with equal amounts of distilled water. Into one having the high original salt content of the culture solution many paramecia were placed, into the other with a very low original salt content few organisms were placed. The paramecia in the first drop died quickly, while in the second drop they lived for days. Obviously the salt content was not the important factor. Here no mention is made of the possible deleterious effects of toxic excreta from so many organisms. If lupine seedlings are grown in distilled water with frequent daily changes the salt content will be kept quite low and in addition the toxic excretions will be more or less eliminated. Under these conditions lupine seedlings grow well until they show signs of injury from lack of nutrient salts. The experiments mentioned above, besides others not reported here, lead us to the belief that the problem of adjustment is so important that it must be taken into account in culture experiments.

THE PROBLEM OF TOXIC EXCRETIONS

For a little more than ten years the Bureau of Soils of the United States Department of Agriculture has sought to explain soil infertility, by the presence of deleterious substances of organic nature in the soils. These substances may be given off by the roots, or arise from

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[15] Frank, B. Untersuchungen über die Ernährung der Pflanzen mit Stickstoff und über den Kreislauf desselben in der Landwirthschaft. Landwirthsch. Jahrb. **17**: 421–553. 1888.

[16] Schulze, E. Über das Verhalten der Lupinenkeimlinge gegen destillirtes Wasser. Landwirthsch. Jahrb. **20**: 236. 1891.

the decay of organic matter. It must be admitted that these investigators have collected a vast amount of facts to substantiate this theory. In Bulletin 23[17] it is shown that the bad characteristics of a soil are transmitted to its aqueous extract. The injury to wheat seedlings grown in this as compared with their growth in an aqueous extract of a good soil, could not be attributed to a difference in nutritive salts for they analyzed practically the same. The conclusion was drawn that the solution contained injurious substances which checked the growth of plants. In Bulletin 28[18] it was shown that wheat seedlings give off substances which are toxic to themselves. This is true when seedlings are grown in nutrient solutions which have been previously used for growing plants. Carbon black or ferric hydrate corrects the used solution so that it produces nearly as good growth as the fresh one. The same is true when distilled water is used instead of the nutrient solution. "These results with distilled water," the author states, "seem to make it very certain that the roots of seedling wheat plants do give off substances which are poisonous to themselves, and these substances can be corrected by carbon black or ferric hydrate." This recalls to mind the experiments by Macaire[19] in 1832, who noted that peas grew poorly in water containing excretions from the roots of the same plants, while wheat on the other hand did well in water charged with the excretions from pea roots. Later, 1845, Boussingault[20] studied the question of root excretion and came to the conclusion that plants do not normally excrete materials from their roots, but may do so in water culture.

For a more extended account of the earlier literature and especially that of the work of the Duke of Bedford and S. U. Pickering on the deleterious effect of grass on fruit trees, the writer wishes to refer the reader to Bulletin 36 by B. E. Livingston, and the works of Schreiner and his assistants, found in bulletins of the Bureau of Soils, U. S. Department of Agriculture, Nos. 47, 53, 70, 74, 75, and 80.

What we are most concerned with at the present time is the possi-

[17] Whitney, M., and Cameron, F. K. Investigations in Soil Fertility. U. S. Dept. Agr. Bur. Soils Bull. 23. 1904.

[18] Livingston, B. E., Britton, J. C., and Reid, F. R. Studies on the Properties of an Unproductive Soil. U. S. Dept. Agr. Bur. Soils Bull. 28. 1905.

[19] Macaire-Prinsep, Memoire pour servir á l'histoire des assolemens; Mém. Soc. Phys. Hist. Nat. Genève, **5**: 282–302. 1832.

[20] Boussingault, J. B. J. D. Rural Economy. Trans. by George Law (London), 345. 1845.

bility of deleterious root excretions from lupine seedlings. We must admit in the beginning that we have not isolated any toxic substance from the water in which these seedlings have been growing. The quantity of poison is obviously so small that it will necessitate the evaporation of 10 or 12 liters of solution to get enough residue for a chemical test. The difficulty of this problem is still further increased since organic chemists can give us no universal method of treatment applicable to all unknown solutions. Previous literature gives us assurance that the excretions are probably organic in nature. The probability is that the substances excreted are compounds of multiple function, and if they do contain an acid radicle, this radicle must be internally compensated with some basic group, since the solution gives no reaction to the ordinary indicators. If an aldehyde is sought for we meet with a difficulty in that we have no differential test to indicate what aldehyde it may be.

On the other hand, we have one or two indirect methods to attack this subject of toxic excretion. After a certain length of time in distilled water lupine roots display certain characteristic features not noticeable in tap water cultures. Roots in tap water are long, slender, grayish white, usually straight and firm. A large conspicuous root cap covers the tip. The water does not become turbid. In distilled water the roots present quite a different appearance. They are covered with a felt-like coating which at first gives the appearance of a mass of root hairs or a weft of fungus mycelium. It is believed that this is nothing more than root cap tissue hanging on the root as the tip pushes forward. On closer examination under the microscope this material does not present the characteristic structure of root hairs or fungus hyphae and resembles in every particular the cells of the outer layer of the root cap. No reason is hazarded for its abundance in distilled water. It has not been noticed in tap water, and appears less abundant in distilled water that has been changed two or three times daily. This weft of tissue falls off and gives the water a turbid appearance. In distilled water the roots are shorter, thicker and usually more or less bent near the tip, showing the tendency for fishhook formation. The lateral roots appear earlier and are more abundant in distilled water cultures than in tap water cultures. They show all the appearances of roots suffering from slight doses of poisons. In all probability the roots excrete a toxin which retards growth. If this toxin is removed by frequent changes of the water the growth rate is increased. This can be shown in gradation. If the water is changed once a day, the growth rate changes but little in the earlier stages of the life of the seedling in the water. If changed twice a day, the growth rate is increased. When changed four times a day the best results were obtained. Again a large number of seedlings growing in the water suffer less apparently than a few in the same amount of water. It seems that the greater amount of salts leached out in one case acts in an antitoxic manner and so corrects the water, or the roots might actually reabsorb some of the salts. Some such explanation might be given for the condition reported by True to the effect "that the leaching process seems to have been somewhat less active in the cultures containing the larger number of roots than in that containing the smaller number of roots." Some such conclusion is more reasonable than to assume that roots under the same external condition do not lose salts at approximately the same rate.

In soils where oxidation is hindered we are told that those substances inimical to growth are more apt to be found. The very nature of water cultures produces this condition of poor oxidation and organic matter collects more rapidly than it can be oxidized. A renewal of the medium in which the plants are growing eliminates to a greater or less extent the toxic substance present therein, and at the same time the roots are living in a solution continually aerated. When an experiment is set up with these facts in mind lupine seedlings will survive in distilled water until injury from lack of nutritive substances sets in. The rate of growth is also greater. Ten seedlings were suspended, as described above, in distilled water. The water

Distilled Water Changed		Distilled Water Not Changed	
Seedling No.	Growth in Mm.	Seedling No.	Growth in Mm.
1	44	1	25
2	34	2	31
3	42	3	31
4	38	4	34
5	32	5	35
6	43	6	37
7	41	7	29
8	37	8	32
9	39	9	36
10	46	10	37
Av. Growth in mm. 39.4		Av.Growth in mm. 32.7	

was changed four times daily. As a control for this experiment 10 seedlings were suspended in another beaker of distilled water. The water in this beaker was not changed. At the end of 30 hours, even, these seedlings surpassed the growth of those left in distilled water without change. This can be seen from the obove table.

The table below gives the data on another experiment extending over a period of four days.

Seedling No.	Distilled Water Changed				Distilled Water Not Changed			
	1st 24 Hrs.	2d 24 Hrs.	3d 24 Hrs.	4th 24 Hrs.	1st 24 Hrs.	2d 24 Hrs.	3d 24 Hrs.	4th 24 Hrs.
	Growth in Mm.	Growth in Mm.	Growth n Mm.	Growth in Mm.	Growth in Mm.	Growth in Mm.	Growth in Mm.	Growth in Mm.
1	27	34	43	46	33	39	43	48
2	34	37	41	45	25	33	40	46
3	37	42	48	55	31	35	36	41
4	33	37	47	55	37	40	43	49
5	32	40	51	58	35	38	43	51
6	35	38	47	55	34	37	42	48
7	31	34	40	45	35	40	43	48
8	37	45	50	60	36	41	45	50
9	38	44	51	61	35	39	41	45
10	38	45	53	60	36	40	42	45
Av. growth in mm........	34.2	39.6	47.1	54.0	33.7	38.2	41.8	47.1

CONCLUSION

In regard to the question as to the toxicity of distilled water, the writer wishes to draw attention to the importance of considering the problem from the dynamic standpoint. To describe how the distilled water produces its effect, it is necessary to know what physical and chemical conditions prevail in the organism and what relation exists between the organism and the medium in which it lives. Our present knowledge of the physical and chemical nature of protoplasm, the basis of all organic life, is too fragmentary upon which to lay the foundation of any theory. However, it seems that one will not stray far away from the truth if he concludes the harmfulness of distilled water due not to any one predominant factor but rather to a resultant of many. These factors may be conceived of as forces bringing about a disturbance of the normal equilibrium of the various chemical and physical interactions within the organism and between it and its environment.

In this paper only two aspects of the subject have been considered.

First, there is the problem of adjustment. Some organisms because of their inherent physiological characteristics possess a low order of resistance to harmful agents and are injured, sometimes fatally, by slight deviations from optimum conditions. However, in the case of lupine seedlings a slow process of adjustment or acclimatization to distilled water fits them better for life within it than if they were thrust into the water immediately. That the change from tap water or culture solution, whatever it may be, to distilled water is great, can be demonstrated by the determination of the electrical conductivity of the waters. It appears that such marked differences disturb the equilibrium between the physical and chemical functions of the organism. Several experiments were made and one was reported to test the theory of adjustment. The conclusion was reached that better root growth was obtained in distilled water when changes from tap to distilled water were made gradually rather than suddenly. This problem of adjustment is so important that it must be taken into account in all cultural work.

The second aspect of the distilled water question dealt with in this paper is that of toxic root excretions. The idea that roots of plants excrete injurious substances, harmful to their further growth, has persisted from the time of De Candolle. The investigations of the Bureau of Soils of the U. S. Department of Agriculture appear to set this theory on a more secure foundation. In this paper are reported the results of experiments to show that, in all probability, seedling roots of *Lupinus albus* excrete a substance that inhibits growth. The toxin was not isolated from the turbid solution but it was shown that when the water was changed four times daily the growth rate of the roots increased. Roots that had grown continually in distilled water presented the appearance of roots injured by dilute solutions of toxic salts. The roots were thick, short, more or less blunt and crooked near the tip, when grown continually in unchanged distilled water. In tap water the roots were long, slender, firm and usually straight. The lateral roots appeared later in tap water than in distilled water.

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AN ANATOMICAL STUDY OF GYMNOSPORANGIUM GALLS

Alban Stewart

Gymnosporangium juniperi-virginianae on Juniperus virginiana

The common "cedar apple" on *Juniperus virginiana* L., caused by *Gymnosporangium juniperi-virginianae* Schw., is one of our most common galls on coniferous plants. While the organism that causes this gall has been widely studied, but little work has been done on the changes brought about in the tissues of the host through the activities of this parasite, and the results obtained are contradictory.

Sanford (8) was the first one to investigate the anatomy of the cedar apple caused by *G. juniperi-virginianae.* He considers the gall to be formed by an abnormal growth of leaf tissue, which carries the apex of the leaf up as the gall develops, and pushes the branch bearing it to one side until the gall appears to be terminal. He further states that the vascular system enters the knot as one single bundle, given off from the vascular bundle of the branch of the tree, and further, that as the knot increases in size the vascular bundle develops rapidly until it appears like the vascular system of a branch.

Wörnle (11) who has done more work than any other investigator on the anatomy of Gymnosporangium galls, considers that the gall arises from a swelling of the stem induced by the fungus, that leaves may also enter into its composition, and, if dormant buds are encountered, they also may be stimulated to development.

Kern (6), who probably bases his statement on the work of Sanford, gives the cedar apple as an example of a gall originating in the leaves. Engler and Prantl (4), on the other hand, describe both *G. juniperi-virginianae* and *G. globosum* as causing woody galls on *Juniperus virginiana.*

Heald (5, pl. 10) has recently figured very young galls of this kind as arising near the axils of the leaves, and Coons (1, figs. 1 and 5) has figured galls some of which appear to be axillary in origin, and others arise from the upper surface of the leaf.

These rather conflicting statements in regard to the morphology of this gall seemed to offer an opportunity for further investigation. As the juniper trees in the vicinity of Madison, Wisconsin, furnished abundant material of this gall in various stages of development I concluded to determine, at least for my own satisfaction, which of these statements was correct. I soon discovered, from external examination, that some of the older galls had remains of more than one leaf attached to them, a fact which in itself would indicate that stem tissue was involved to a greater or less extent. I found still further, that normal branches are attached to the gall, apparently growing out from it at times (fig. 7), a condition that would be rather unusual if the gall were composed entirely of modified leaf tissue. Rather thick hand-sections showed that when a gall was located on the end of a stem, the stem continued into it for some distance before it broke up. This hasty examination seemed to justify a more careful study, the results of which are given in the following pages.

Before entering into the description of the anatomy of the gall it seems desirable to describe briefly the anatomy of both the leaf and young stem, as both of these are involved in gall formation.

The normal leaf is triangular in cross section except towards the base where it is rather four-sided. The epidermis is heavily cutinized and forms a smooth layer on the outer (morphologically lower) side of the leaf. Towards the edges and inner side, however, the individual cells are somewhat raised towards the outside causing them to appear more or less papilliform when seen under the microscope. The stomata occur mostly in the inner (morphologically upper) epidermis, but they may appear occasionally in the outer epidermis near the edges of the leaf.

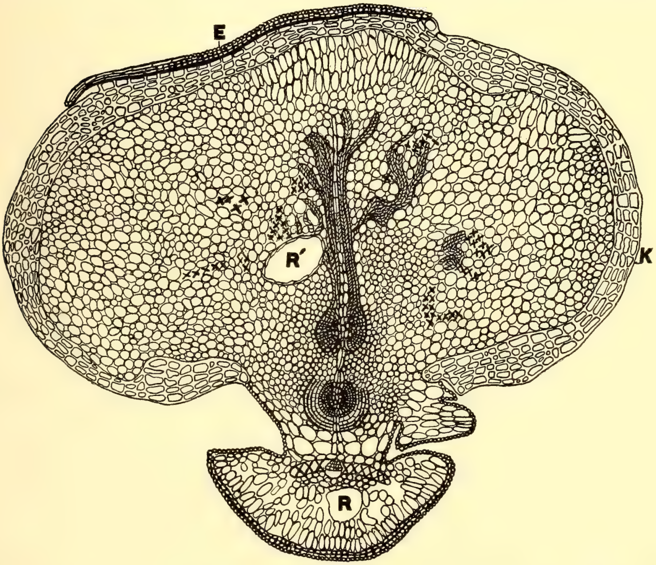
In addition to the epidermis, the outer side of the leaf is protected by a hypodermis which may extend entirely or only part way across this side. The hypodermal cells are usually arranged in one or two layers, although three layers may be present at times over a space. Sanford (8) describes a double epidermal layer for this side of the leaf but he evidently mistook the hypoderm for an inner epidermal layer. The interior of the leaf is filled with rather thin-walled parenchyma cells (text fig. 1) which are loosely arranged towards the inner side near where the stomata are borne, and resemble very much ordinary spongy leaf tissue. Towards the outer side of the leaf the parenchyma cells are elongated perpendicular to the surface and form a palisade tissue. The base of the leaf is united with the stem for some distance. A large resin cavity occurs in this region of the leaf. A single bundle enters the upper part of the leaf, xylem above and phloem below. On each side of this bundle are peculiar tracheids with bordered pits and bar-like thickenings (text fig. 1, xxx). For further consideration of these tracheids see De Bary (2).

It is sometimes difficult to determine in the young stem which of the cells belong to the leaf and which to the outer bark of the stem as the leaf and stem tissue blend together more or less at the point of union of leaf and stem. Between the attachment of the opposite leaves, however, the stem is exposed and is covered with epidermal cells similar to those which occur on the inner side of the leaf. There is a single layer of rather thick-walled parenchyma cells inside the epidermis, followed farther in by one or more layers of large, thin-walled, oval-shaped parenchyma cells. The inner bark is composed of concentric rings of bast fibers between each two of which there are three layers of cells. According to De Bary (2), two of these layers are of sieve tubes which are separated from each other by a layer of parenchyma cells.

The xylem of the young stem is composed of small triangular groups of tracheids, separated from similar adjacent groups by uniseriate rays. The rays extend outward into the inner bark, thus dividing the phloem and bast region into segments. The rays are broader opposite where the leaf trace bundles leave the xylem portion of the central cylinder. This broadening comes about usually by an enlargement of the ray cells, but sometimes there is an interpolation of additional ray cells. As two leaf traces leave the stem about opposite each other, the gaps caused by the broader rays divide the woody portion of the central cylinder into two nearly symmetrical halves. A small V-shaped depression occurs in the xylem where the leaf trace leaves it. A small group of pith cells occupies the center of the stem.

A diagrammatic drawing of a very young cedar apple gall and the stem that bears it is shown in figure 1. This gall has arisen from the axil of a leaf, a portion of which is shown to the right of the figure. I have examined a large number of young galls and have been unable to find any which were similar to the two figured by Coons (1, right side of fig. 1), which according to the figure have arisen evidently from the surface of the leaf. I have also been unable to find any young galls which were terminally located, and which might have arisen from the infection of a terminal bud such as has been suggested by Wörnle. They were always distinctly axillary in position although on the same tree with them there were older galls which were apparently terminal. According to Sanford (8), the branch is pushed to one side during the development of the gall. If this be true the terminal position of many of the older galls is probably more apparent than real.

The leaf, from the axil of which the gall arises, is pushed outward to some extent so that a small shelf is formed on which the gall rests. Apparently the fungus has not entered the stem at this stage as no abnormalities appear beyond a slight production of cork just above the gall (fig. 1). In the stage figured, the gall consists entirely of



TEXT FIG. 1. Semi-diagrammatic drawing of a cross section of a young gall of *Gymnosporangium juniperi-virginianae* and of the stem that bears it. Normal leaf shown below with resin cavity (*r*). Immediately above this is the central cylinder of the stem, and above this the central cylinder in the gall with its branches. *E*, epidermis and hypodermis of leaf; *K*, cork layer surrounding gall; *R′*, resin cavity in gall; × 42.

rows of parenchyma, which is covered on the outside by layers of cork cells (*k*). The leaf trace bundles are shown at *l*, two of which leave the woody cylinder (*c*), about opposite each other. Immediately above the leaf-trace bundle, on the right side of the figure, there is another bundle which is given off from the woody cylinder of the stem and extends to the base of the gall. Figure 2 is a diagrammatic drawing of a longitudinal section of a young stem and of an axillary bud (*bd*). Towards the lower part of the figure the central cylinder gives off two leaf-trace bundles (*l*), which go to a pair of opposite leaves. Just above the place of departure of the leaf-trace bundles, the central cylinder of the stem divides. One of the branches goes off to the right as a continuation of the stem, while the branch to the left enters the base of the young bud (*bd*). The similarity between figs. 1 and 2 is so great as to leave no doubt about the origin of the gall, as the vascular systems are the same in each case. The gall can be nothing more than an abnormally developed axillary bud, but whether or not it is a bud transformed I am unable to state definitely. It seems likely that the fungus has stimulated the development of a bud which from the start is transformed into a gall. I have examined a number of galls, in about the same stage of development as the one shown in figure 1, but have failed to find in any of them an indication of embryonic leaves, such as would probably be the case if the bud were already formed when the infection took place. In the witch's broom on this same species of juniper, caused by *Gymnosporangium juvenescens* Kern, the development of buds is greatly stimulated. It is not unreasonable to suppose that a somewhat similar condition exists here in regard to bud development.

After having considered to some extent a longitudinal section of the vascular system of a very young gall, it seems well to consider the cross section of a somewhat older gall and of the branch that bears it. Such a section is shown semidiagrammatically in text figure 1. A cross section of a normal leaf is shown in the lower part of the figure with a leaf trace bundle entering it. The leaf trace is flanked on each side by rows of tracheids with bar-like thickenings, shown by ××× in the figure. A portion of the base of another leaf is shown to the right, while the gall occupies the upper part of the figure. Just above the section of the lower leaf is the cross section of the stem that bears the gall. The heavy lines represent the rings of bast fibers, which are absent on the side next to the gall. Above the cross section of this stem there is still another, which in lower sections has all of the structures of a normal stem. There are triangular wedges of xylem, leaf gaps, rays somewhat broader than normal, and a pith. Surrounding this is a region of secondary bark tissue resembling the normal in many respects except that the bast rings are poorly developed. In the section drawn the woody portion of the cylinder has become more or less broken up and the cells turned over but it still preserves enough of the original structure to show that it is a stem. In place of secondary bark tissue there are small parenchyma cells instead. It should be noticed that a bundle leaves this modified stem on the upper side and extends into the gall. It is made up of two strands of scalariform tracheids, separated from each other the most of the way by a strand of parenchyma. This bundle is too large for a leaf-trace bundle, nor does it have the structure of such. It is more like that of a branch the central strand of parenchyma probably being the pith. Branches are given off from this which run an irregular course. These branches are composed of scalariform tracheids which often have groups of other tracheids with bar-like thickenings in connection with them shown by ××× in the figure. Groups of similar tracheids occur in other parts of the gall. The epidermis and hypodermis of the leaf is shown at *e*, on the upper side of the figure. This is the leaf from the axil of which the gall has arisen (see fig. 1) the tissue of which has become involved in the formation of the gall since the stage shown in the figure just mentioned. The gall is surrounded by several layers of rather large cork cells. The remainder of the gall is of moderately thin-walled parenchyma, the cells of which vary greatly in size. What is evidently a part of the palisade tissue of the leaf is still preserved in the upper part of the gall, just inside the epiderm and hypoderm (*e*). The remains of the old resin cavity of the leaf is shown at *r′*.

I have examined serial sections of a number of galls in about the same stage of development as the one shown in text fig. 1, and have always found essentially the same structures. It was not always the case that as good a transverse section of the stem inside the gall was seen as the one shown in the figure, as that depends on the plane through which the sections are cut, and is largely a matter of chance. I was always able, however, to get enough in the sections to show that such a structure was present. The epidermis and hypodermis of the old leaf always appear, and usually some of the palisade tissue. The cork layer usually forms on the outside, but one gall was found in which it had started to form below the periphery, cutting off some of the parenchyma. Stages between those shown in fig. 1 and text fig. 1, show that the tissue towards the base of the leaf first becomes involved in gall formation. The tissue towards the base may be greatly altered while that at the apex is still normal. The resin cavity may become very much elongated so that it appears at a higher level in the gall than it would in the normal leaf.

After having found a distinct stem structure in the young galls it is interesting to see what becomes of it in older galls. Fig. 3 is a diagrammatic drawing of a cross section made near the base of a gall near where the stem enters it. The stem has not become entirely enclosed by gall tissue at this point as a portion of the cork of the stem is still shown at *k*. The secondary bark tissue preserves many of its normal characters. The bast fibers occur in concentric rings, and there are sieve tubes and parenchyma layers between them. The bast rings are not continuous, however, as some of the fibers have been converted into parenchyma cells, a condition not dissimilar to what De Lamarlière (3) describes for the galls of *Gymnosporangium clavariaeforme* on stems of *Juniperus communis*. Bast fibers are entirely absent on the upper left side of the figure, while on the lower left side they are turned over nearly at right angles to their usual upright position. Occasional bast fibers are formed in the layers where normally only sieve tubes and parenchyma are developed. This is evidently not due to fibers being pushed out of place as they may occur where the bast rings are unbroken (see upper right side of figure).

The structure of the xylem is nearly normal except towards the upper side of the figure. The tracheids are here turned over more or less, and two multiseriate rays have formed in the wood.

There is not much change in the woody portion of the cylinder for some distance farther in from the section shown in fig. 3. Two projections appear after a time which develop into the condition shown in fig. 4. A large pith has developed in the center and two branches extend above and below. The one in the upper part of the figure is quite regular and presents many of the structures of a stem. A pith in the center is surrounded by wood in which there are rays extending outward into the somewhat modified secondary bark tissue (*b*) in which there are a few bast fibers distributed. The branch, which extends off on the lower side, is less regular, and soon breaks up into irregularly running strands of tracheids. The only parts which preserve a transverse stem structure are to the right and left of the center of the figure. There are a few wedges of xylem here which are separated by rays which are sometimes broader at the inner ends of the wedges. There are strands of tracheids running away from the central portion which are much bent and twisted. They extend in various directions. Some of them have tissue similar to the secondary bark of a normal stem, so they probably represent branches from the main central stem of the gall, but very much distorted and broken up. The tracheids which compose the irregular strands of vascular tissue usually have scalariform pits in their walls. The thickenings between the pits are often so slender as to give the appearance of spiral tracheids.

In addition to the irregular strands of tracheids that have just been mentioned, peculiar stem-like structures occur in the gall. These often present a true transverse stem structure so there can be no mistake about their being stems. As the origin of these is better shown in the next species to be considered, they will be taken up in that connection. Two of these are shown at *s*, in figs. 4 and 5. They are much more irregular in structure here than is often the case.

By further breaking up of the central cylinder deeper in the gall, a condition is finally reached in which there is nothing left but irregularly running strands of fibrovascular tissue. This condition is shown rather diagrammatically in fig. 5. All semblance of a transverse stem structure has disappeared, except towards the lower left side of the figure, and in two small areas in other places. The strands have become so twisted, in the central part of the figure, that the modified secondary bark tissue lies towards the center. Strands of tracheids sometimes surround rather broad ray-like masses of parenchyma as is shown in the upper part of the figure. From the central body, as figured, strands of tracheids radiate outward towards the periphery of the gall.

Sanford (8) makes the statement that there is but little distinction between xylem and phloem in the smaller bundles of the gall. The tracheids in the smaller bundles often show less lignification than those of the larger bundles, so that it is often difficult to differentiate sharply the tissues by staining. It is usually possible by careful staining, however, to show that certain of the cells are slightly lignified and have scalariform pits in their walls. Furthermore, elongated parenchyma cells usually accompany these in such a relation that one may assume that they are phloem cells, or cells that perform a similar function. Cells sometimes occur which are part parenchyma and part tracheid. They occur quite commonly in the gnarl-like complexes of cells in various places. As they are shown better in the next species, they will be considered further in that connection.

After having considered stages in the breaking up of the stem in the gall as seen in transverse section, a longitudinal section will now be considered. Such a section is shown diagrammatically through the basal portion of a mature gall in fig. 6. The central cylinder of the stem which bears the gall is shown at *c*, which continues off at the upper left side of the figure. The two branches to the right constitute the central cylinder which enters the gall. This cylinder has a very broad pith (*p*) which begins very suddenly near where it joins the main stem. The bundles on each side of the pith have a tissue similar to secondary bark (*b*), in which there are strands of bast fibers. Rays occur among the tracheids of both bundles, and continue into the secondary bark as shown by the cross lines in the drawing. A portion of the old leaf is still attached to the lower part of the gall, some of the tissue of which seems still to be unaltered. The epidermis and hypodermis are shown at *e*, and the resin cavity at *r*, surrounded by the more or less modified parenchyma of the leaf. Immediately above the resin cavity there is a strand of tracheids with bar-like thickenings (*l*), such as always accompany the leaf-trace bundles. This branches and joins the base of the central cylinder entering the gall at about the same place that the leaf-trace bundle joins the central cylinder in the young gall shown in fig. 1. There can be no doubt about this being a portion of a leaf-trace bundle especially as another leaf trace (*l′*) leaves the central cylinder just opposite. There is an irregular mass of tracheids just above this strand, but whether or not it is made up of tracheids from the leaf trace, or is a branch from the main stem, I could not determine. The leaf trace bundle, in addition to the other bundles, is thus accounted for in a very young as well as in an old gall; so there can be no further doubt that the other bundles are derived from a stem. They are not leaf-trace bundles which have assumed the structure of a stem as was stated by Sanford.

The remainder of the gall tissue consists of loosely arranged parenchyma cells which are surrounded on the outside by layers of cork cells. Except near the central cylinder, the parenchyma cells for the most part are not strikingly larger in the older gall than they are in the younger gall (shown in text fig. 1). Starch as well as droplets of oil occurs abundantly in many of the cells.

Since this article was sent away for publication a paper has appeared by Reed and Crabill (12) in which the anatomy of the gall of *Gymnosporangium juniperi-virginianae* is briefly considered. These authors regard this gall as arising from nothing more than an hypertrophied leaf, and that its fibrovascular system is a modified continuation of the fibrovascular system of the leaf.

From the descriptions and figures which have been given in the preceding pages, it seems evident that these authors have been mistaken in their interpretations. The diagrammatic drawing of a longitudinal section of a young gall and of a branch, which they show in their fig. 4, is probably of a stage intermediate between the galls I have figured in fig. 1, and text fig. 1. These authors have seemingly failed to find a leaf-trace bundle in addition to what they have shown, and have interpreted the fibrovascular system as a modified leaf trace. A section cut at right angles to the one shown in their figure would probably reveal the fact that this system has the structure of a stem and is in no way a leaf bundle.

Gymnosporangium globosum on Juniperus virginiana.

The relation of the younger galls of *G. globosum* Farl. to stem and leaf is very similar to that of *G. juniperi-virginianae*, considered earlier in this article. In fact the similarity is so great that it is practically impossible to distinguish the two with any degree of certainty until the sori have formed. Cross sections of the younger galls have essentially the same structure as galls of similar size which are caused by *G. juniperi-virginianae* (text fig. 1). There is this exception, however, that the strands of tracheids given off from the stem in the gall, which correspond to the two strands at the right of the resin cavity (*r′* in the above mentioned figure), are separated by a very broad band of parenchyma. This condition is to be expected from what is usually found in the older galls caused by this species of Gymnosporangium.

The woody cylinder in older galls presents two entirely different types as far as was shown by the material studied. The more common of these types will be considered first. A cross section of an oldegall, made near where the gall joins the main stem, is shown diagrammatically in fig. 8. The relative position of this section in the gall is about the same as the section of *G. juniperi-virginianae* shown in fig. 3. The magnification of this figure is much higher than is that of fig. 8, so that the central cylinder is much wider at the start in the *globosum* gall. What is evidently very much modified tissue of the secondary bark is shown at *b*, which is not well differentiated on the right and left sides of the figure. Bast fibers are fewer in number as can be seen by comparing the two figures just mentioned. A median longitudinal section of a portion of the stem bearing the gall is shown at *c*, the pith being the stippled portion in the center. Tracheids radiate outward above and below, and in the center of the figure they take an irregular course next to the secondary bark tissue. Sections taken a little deeper often show strands of tracheids running through the modified pith tissue (fig. 9) which often surround broad, ray-like masses of parenchyma. They may continue for some time and then disappear resulting in the condition shown in fig. 9. Secondary bark tissue is fairly well differentiated in this section and can be seen entirely around the cylinder. The tracheids take a very irregular course inside of this, but in places there are wedges of xylem which show a true transverse structure. They are shown by the triangular area covered with cross lines in fig. 9, and one is shown under high magnification in fig. 12. This wedge presents what appears to be three distinct rings of growth, as there are three bands each of spring and summer wood alternating with each other. Whether or not these are true annual rings I do not know. As this gall is perennial in growth, and as secondary thickening takes place in both of the galls being considered in this article, it is not unreasonable to suppose that they are true rings of growth. If they are rings of growth they are better developed, where they occur, than in the galls of *G. juniperinum* where, according to Wörnle (11), growth rings are poorly marked.

The woody cylinder widens deeper in the gall as can be seen by comparing figs. 8 and 9. The greatest diameter in the gall figured is nearly 5 mm., but galls were examined in which it was more than 8 mm. The cylinder finally breaks up and branches out in the gall, but preserves a semblance of a stem structure much deeper than in the gall of *G. juniperi-virginianae* previously considered.

The origin of secondary stem structures in the gall is shown in figs. 13, 14, and 15. A portion of the woody cylinder (fig. 9), pushes outward in the manner shown in fig. 13. This projection becomes larger and somewhat expanded at the apex while it contracts at the base (fig. 14). Strands of tracheids, surrounding broad, ray-like masses of parenchyma, often fill up the constricted portion as is shown in the figure. After a time the projection becomes separated from the central cylinder, or nearly so (fig. 15), after which it assumes the character of the main woody cylinder from which it arose, except that it is smaller in size.

I have been unable to determine the cause of these accessory stems, but, arising as the gall does from a transformed axillary bud, it is not unreasonable to suppose that latent properties for bud formation, inherent in it, are stimulated to activity through the action of the fungus.

A longitudinal section through this type of gall is shown in fig. 10. The section figured was made at right angles to the ones shown in figs. 8 and 9. A portion of the stem which bears the gall is shown at *c*. Strands of tracheids go off from this on the right and left sides of the figure. This is surrounded by gall parenchyma (*gp*) which is surrounded on the outside by cork (*k*).

In the second type to be considered, the woody cylinder preserves more of its normal structure at the point of entrance into the gall, and is more like the galls of *G. juniperi-virginianae* in this respect than in the type just described. The woody cylinder is not greatly altered at first, but there is a considerable production of parenchyma at the expense of the tracheids. Many of the parenchyma cells have the form of tracheids when seen in cross section, but the walls are unlignified and protoplasm is present in them. Broad rays are formed which cut the cylinder into wedge-like masses similar to the one shown in fig. 12. Bast fibers occur but seldom in the secondary bark tissue, similar in this respect to the first of the type of *globosum* gall described.

A short distance beyond the point of entrance of the stem into the gall, the pith begins to enlarge and the rays broaden, thus pushing the xylem wedges farther apart. This process is attended with more or less displacement of tracheids in sectors of the cylinder. The enlargement of the pith and other abnormalities continue, until deep in the gall the woody cylinder assumes a form very similar to that shown in fig. 9, where most of the groups of tracheids extend in various directions. A few of them still remain in their normal position and appear as the xylem wedges, mentioned several times before in this article. Sooner or later the various groups of tracheids diffuse outward into the gall and all semblance of a central stem structure is lost.

Gnarl-like complexes of tracheids and parenchyma cells occur in both of the types of globosum galls described. The one shown in fig. 11 consists of a mass of cells wound around a parenchyma center and is not dissimilar to the "Knaueln" described by Mäule (7) from traumatic wood. Cells which are transitional between tracheids and parenchyma are shown in this figure. One end of such a cell usually has a lignified wall in which there are scalariform pits, while at the other end the wall is of cellulose and a protoplasmic content is present. Transitional cells of this nature have been reported in several other kinds of pathological plant tissue. Mäule (7) has found them in the traumatic wood of *Abies cephalonica*; Smith (9) has described and figured somewhat similar cells in the tumor strand of crown gall on the tobacco stem; and the writer has found such cells to be present in the galls of *Peridermium cerebrum* on *Pinus Banksiana* (see Stewart (10)). Such cells have probably been reported in still other kinds of abnormal plant tissue. They seem to have quite a wide distribution.

METHODS

In preparing material for study, the galls were embedded in celloidin as there was too much lignified tissue present to get good section when embedded in parafin. The sections were usually mounted in series and stained with safranin. Either Delafield's hematoxylin or "licht Grün" was used as a counter stain. The drawings were made with a Spencer Delineascope and are diagrammatic for the most part. In order to cover wide enough field to show all of the structures desired, a low magnification was necessary. Great detail was impossible under such circumstances.

SUMMARY

1. *Gymnosporangium juniperi-virginianae* and *G. globosum* cause the formation of large galls on the younger branches of *Juniperus virginiana*.

2. The galls arise from the axils of the leaves and are evidently transformed axillary buds.

3. Young galls have two distinct fibrovascular systems, one of which is a leaf-trace bundle, and the other a stem.

4. The more or less modified stem which enters the base of the older galls gradually breaks up and radiates outward, deeper in the gall tissue.

5. Leaf tissue is also involved in gall formation, and remains of it are usually to be found adhering to the older galls.

6. Normal stems sometimes appear to have grown out from the surface of the older galls.

7. Accessory stem structures occur, which probably originate by a branching of the main stem in the gall.

8. Broad, ray-like masses of parenchyma, surrounded by tracheids, are of rather common occurrence.

9. Irregularly twisted masses of fibrovascular tissue occur which are similar in many respects to like structures in traumatic wood.

10. Cells which are transitional between parenchyma and tracheids are quite abundant.

11. The irregularly running bundles in the gall are composed largely of scalariform tracheids.

12. The statements in the above summary apply in a general way to both the galls considered in this article.

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EXPLANATION OF PLATES XV AND XVI

PLATE XV

Gymnosporangium juniperi-virginianae on *Juniperus virginiana*

FIG. 1. Diagrammatic drawing of a longitudinal section through a very young gall and of the stem that bears it. *C*, central cylinder of stem. *E*, epidermis and hypodermis of leaf. *K*, cork layer. *L*, leaf trace bundles. *R*, resin cavity in leaf; × 42.

FIG. 2. Diagrammatic drawing of a longitudinal section of a portion of a young juniper stem and an axillary bud. *Bd*, axillary bud. Other lettering same as in figure 1; × 42.

FIG. 3. Diagrammatic drawing of a cross section of a stem near where it enters the base of the gall. *B*, tissue of secondary bark. *K*, cork layer; × 42.

FIG. 4. Similar to fig. 3, but taken from a section deeper in the gall. *B*, modified tissue of secondary bark. *P*, pith. *S*, accessory stem structure in the gall; × 42.

FIG. 5. Similar to fig. 4, but taken from a section still deeper in the gall where the stem has become almost entirely disorganized. Lettering same as in fig. 4; × 42.

FIG. 6. Diagrammatic drawing of a longitudinal section through the basal portion of an older gall and of the stem that bears it. *B*, modified tissue of secondary bark. *C*, central cylinder of main stem bearing gall. *E*, epidermis and hypodermis of leaf which has become involved in gall formation. *L*, leaf-trace bundle. *P*, pith of central cylinder inside gall. *R*, resin cavity in leaf; × 42.

FIG. 7. Apparently normal branch growing from gall. *B*, more or less modified tissue of secondary bark. *K*, cork layer surrounding gall. *R*, resin cavity in leaf; × 14.

PLATE XVI

Gymnosporangium globosum on *Juniperus virginiana*

FIG. 8. Diagrammatic drawing of a cross section of the central cylinder near where it first enters the gall. *B*, modified tissue of secondary bark. *C*, portion of central cylinder of stem that bears the gall. *Gp*, gall parenchyma which entirely surrounds the parts figured; × 14.

FIG. 9. Similar to fig. 8, but from a deeper section in the gall. *P*, pith. Other lettering same as in fig. 8; × 14.

FIG. 10. Diagrammatic drawing of a longitudinal section through a gall made at right angles to the ones shown in fig. 8 and 9. The figure shows a longitudinal view of the central cylinder at its point of entrance into the gall. *K*, cork layer. Other lettering same as in figures 8 and 9; × 14.

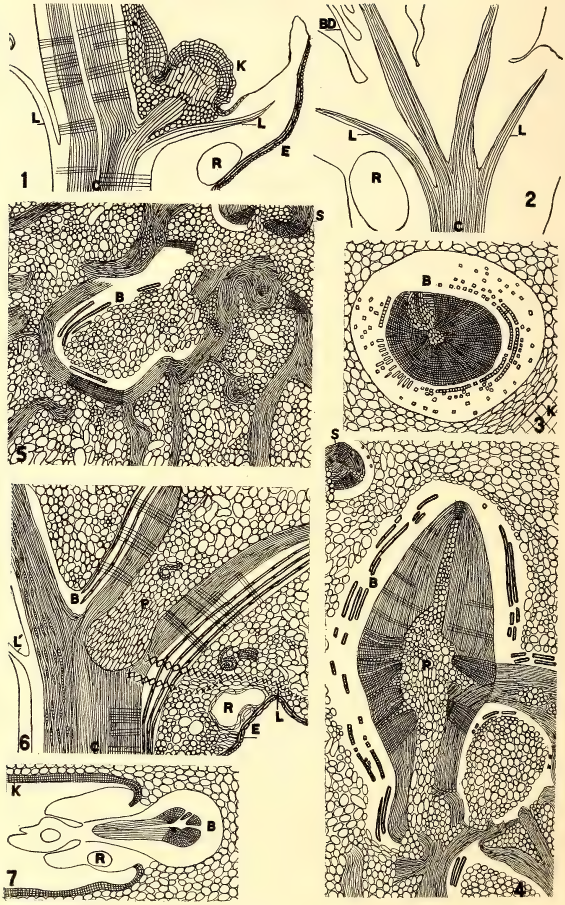
FIG. 11. Drawing of one of the gnarl-like complexes of cells in the gall, showing scalariform tracheids, parenchyma cells, and cells which are transitional between tracheids and parenchyma; × 200.

FIG. 12. Drawing of a cross section of one of the xylem wedges in the gall with three rings of growth. Similar wedges are shown diagrammatically in figs. 9, 13, 14, and 15; × 200.

FIG. 13. Pushing outward of a portion of the woody part of the central cylinder in the gall preparatory to forming an accessory stem structure; × 14.

FIG. 14. Similar to fig. 13, but showing a somewhat later stage; × 14.

FIG. 15. Accessory stem structure near point of departure from main central cylinder in gall; × 14.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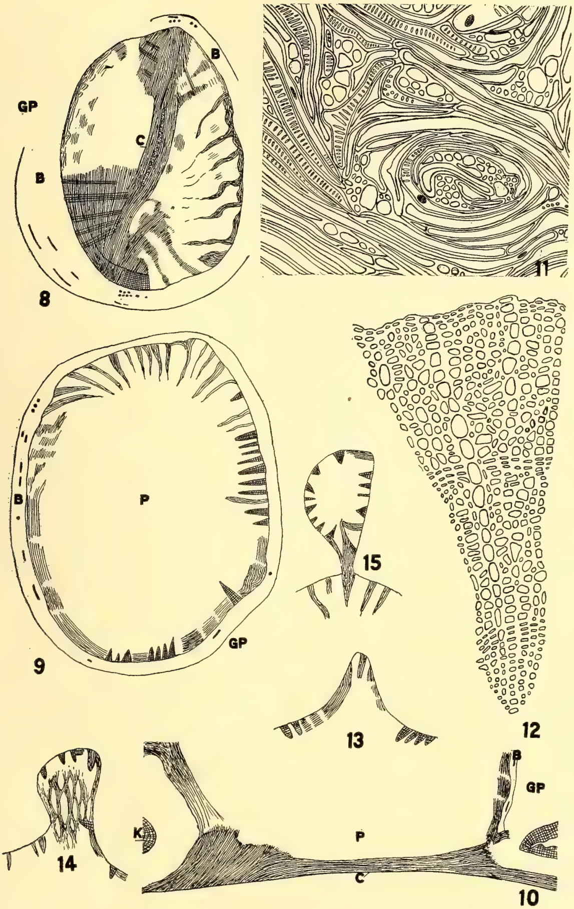
STEWART: GYMNOSPORANGIUM GALLS.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STEWART: GYMNOSPORANGIUM GALLS.

AN EXTENSION TO 5.99° OF TABLES TO DETERMINE THE OSMOTIC PRESSURE OF EXPRESSED VEGETABLE SAPS FROM THE DEPRESSION OF THE FREEZING POINT

J. ARTHUR HARRIS

Some months ago Dr. Gortner and I published in this journal (Amer. Journ. Bot. **1**: 73–75, Feb. 1914) tables of the value of

$$P = 12.060\Delta - .021\Delta^2$$

for freezing point lowerings of .01° to 2.99°.

At that time this range of values seemed to us, as well as to others,

Δ	Hundredths of Degrees, Centigrade									
	0	1	2	3	4	5	6	7	8	9
3.0	35.99	36.11	36.23	36.35	36.47	36.59	36.71	36.83	36.95	37.06
3.1	37.18	37.30	37.42	37.54	37.66	37.78	37.90	38.02	38.14	38.26
3.2	38.38	38.50	38.62	38.73	38.85	38.97	39.09	39.21	39.33	39.45
3.3	39.57	39.69	39.81	39.93	40.05	40.17	40.28	40.40	40.52	40.64
3.4	40.76	40.88	41.00	41.12	41.24	41.36	41.48	41.60	41.71	41.83
3.5	41.95	42.07	42.19	42.31	42.43	42.55	42.67	42.79	42.91	43.02
3.6	43.14	43.26	43.38	43.50	43.62	43.74	43.86	43.98	44.10	44.22
3.7	44.33	44.45	44.57	44.69	44.81	44.93	45.05	45.17	45.29	45.41
3.8	45.52	45.64	45.76	45.88	46.00	46.12	46.24	46.36	46.48	46.60
3.9	46.71	46.83	46.95	47.07	47.19	47.31	47.43	47.55	47.67	47.79
4.0	47.90	48.02	48.14	48.26	48.38	48.50	48.62	48.74	48.86	48.97
4.1	49.09	49.21	49.33	49.45	49.57	49.69	49.81	49.93	50.04	50.16
4.2	50.28	50.40	50.52	50.64	50.76	50.88	50.99	51.11	51.23	51.35
4.3	51.47	51.59	51.71	51.83	51.94	52.06	52.18	52.30	52.42	52.54
4.4	52.66	52.78	52.89	53.01	53.13	53.25	53.37	53.49	53.61	53.73
4.5	53.84	53.96	54.08	54.20	54.32	54.44	54.56	54.68	54.79	54.91
4.6	55.03	55.15	55.27	55.39	55.51	55.62	55.74	55.86	55.98	56.10
4.7	56.22	56.34	56.46	56.57	56.69	56.81	56.93	57.05	57.17	57.29
4.8	57.40	57.52	57.64	57.76	57.88	58.00	58.12	58.23	58.35	58.47
4.9	58.59	58.71	58.83	58.95	59.06	59.18	59.30	59.42	59.54	59.66
5.0	59.78	59.89	60.01	60.13	60.25	60.37	60.49	60.60	60.72	60.84
5.1	60.96	61.08	61.20	61.32	61.43	61.55	61.67	61.79	61.91	62.03
5.2	62.14	62.26	62.38	62.50	62.62	62.74	62.85	62.97	63.09	63.21
5.3	63.33	63.45	63.56	63.68	63.80	63.92	64.04	64.16	64.27	64.39
5.4	64.51	64.63	64.75	64.87	64.98	65.10	65.22	65.34	65.46	65.58
5.5	65.69	65.81	65.93	66.05	66.17	66.29	66.40	66.52	66.64	66.76
5.6	66.88	67.00	67.11	67.23	67.35	67.47	67.59	67.71	67.82	67.94
5.7	68.06	68.18	68.30	68.41	68.53	68.65	68.77	68.89	69.01	69.12
5.8	69.24	69.36	69.48	69.60	69.71	69.83	69.95	70.07	70.19	70.30
5.9	70.42	70.54	70.66	70.78	70.90	71.01	71.13	71.25	71.37	71.49

quite sufficient for biological work. Since then, however, Mr. John V. Lawrence and I in determinations of freezing point depressions on the juices of the plants of the southwestern deserts about Tucson and of the Jamaican coastal deserts have repeatedly found values far beyond the range of the table there published.[1]

It has seemed worth while therefore to extend the tables to 6°.

In using such a table one must of course remember the difficulties which surround the problem of the osmotic pressures of concentrated solutions—even those of pure solutes in pure solvents under the controlled conditions of the physico-chemical laboratory. But the physiologist cannot neglect the many problems presented by the concentration of vegetable saps until physical chemists are in full agreement concerning all details. Quite to the contrary he must use the best methods he can under the peculiarly complex and difficult conditions which surround his work and express his results in consistent terms, remembering that methods and formulae which are the best that are practicable at present may soon have to be replaced by others.

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[1] These results confirm those obtained in cryoscopic studies by Cavara and by plasmolytic methods by Fitting, in that they show that very high concentrations may occur in plants growing in their natural habitats. As yet we have not found concentrations quite so high as some they report.

CALCIUM HYPOCHLORITE AS A SEED STERILIZER*

James K. Wilson

For certain physiological experiments seeds and plantlets free from active bacteria and fungi are necessary. Most attempts to secure such seeds or plantlets in any considerable number have resulted in failure, usually because of the harmful effects of the germicide, its low efficiency, or the complicated methods required for treatment of the seed. While it is recognized that no germicide will give perfect satisfaction under every condition, some are more effective in this respect than others.

The treatment of seed to remove bacteria and fungi, especially the latter, has been practiced for a considerable period of time; and while one investigator has secured fair results with a particular method, another one has considered it a failure, or nearly so, when tested on another kind of seed. As a result many methods have been proposed for seed sterilization with the final condition that most of them can not be relied upon to yield a very large percentage of sterile plantlets.

In the following compilation the methods of treatment employed by various investigators are summarized together with the seeds treated.

Author	Seed	Disinfectant
Brown and Escombe.	*Hordeum vulgaris.*	$CuSO_4$, 1%, one to two hours.
Combes.	Radish.	$HgCl_2$.
Czapek.	Corn.	$HgCl_2$, 1%, two minutes.
Godlewski and Polzeniusz.	*Pisum sativum, Vicia Faba, Triticum vulgare, Zea mays, Ricinus communis, Brassica napus.*	$HgCl_2$, 1/100%.
Grüss.	Grain.	HOH 48 hours. Then in $HgCl_2$ 45 minutes.
Hansteen.	*Zea mays* and others.	Washed in absolute alcohol then in $HgCl_2$ 1–1,000.
Kehler.	Peas, beans, blue lupine, yellow mustard, red clover, sugar beet, wheat, alfalfa, and others.	$HgCl_2$, CH_2O, various strengths of each.

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* Contribution, Laboratory of Plant Physiology, Cornell University.

Laurent.	Corn, peas, lentils and other seeds.	$HgCl_2$ 1–500 alone and in combination with other substances.
Lefevre, J.	*Lepidium sativum, Ocimum minimum, Tropaeolum nanum.*	$HgCl_2$, 2 to 3 pts. per 1,000, 15 min. and longer.
Lewis and Nicholson.	Leguminous seed.	Phenol 5%, 50 min.
Lutz, L.	*Cucurbita maxima, Zea mays, Cucumis prophetarum, Helianthus annus* and others.	$HgCl_2$ 1–2,000, 5 min.
Maze and Perrier.	*Zea mays.*	$HgCl_2$ 1–1,000, 15 min.
Molliard, M.	*Raphanus sativus, Allium cepa, Ipomaea purpurea, Nasturtium officinale.*	Soak seed in sterile water until wrinkled then in abs. alc. for a short period, then 1 min. in 1% $HgCl_2$.
Nabokich, A.	*Zea mays* and others.	Bromine water 1–1,000 20 to 30 min.
Nobbe, F.	Wheat, timothy and others.	$CuSO_4$ various strengths for 24 hours.

AUTHOR	SEED	DISINFECTANT
Pinoy and Magrou.	*Sinapis alba, Lupinus albus, Zea mays, Orobus tuberosus.*	Br. water and H_2O_2.
Polowczow, W.	*Lepidium sativum, Lupinus luteus* and others.	Br. water.
Prazmowski, A.	Garden peas.	$HgCl_2$ 2%, then absolute alcohol and burned off alcohol.
Puriewitsch, K.	Endosperm of *Zea mays, Triticum sativum, Secale cereale, Hordeum distichum, Oryza sativa* and others.	CH_2O, 2½ parts—1,000, 3 min.
Robinson, T. R.	Alfalfa, crimson clover, garden pea, soy bean, wheat, oats, radish.	CH_2O, $HgCl_2$, H_2O_2 in various strengths.
Schroeder, H.	Wheat.	$AgNO_3$ 5%, 14 hrs.
Schroeder, H.	Wheat.	HNO_3, $AgNO_3$, $HgCl_2$, and other substances in combination.
Schulow, Iw.	Corn, peas, barley.	Br. water 20 min.
Staklasa, J.	*Hordeum distichon.*	$HgCl_2$ 0.1%.
Stoward, F.	*Hordeum, Zea* and *Ricinus.*	$CuSO_4$, $HgCl_2$, CH_2O, Sat. sol. chloroform. Sat. sol. toluene.
v. Ubisch, G.	Spore capsules of moss.	Alcoholic $HgCl_2$.
de Zeeuw, R.	*Lupinus albus, Pisum sativum, Triticum vulgare, Hor-*	H_2O_2, $HgCl_2$, cleaning fluid. Potassium dichromate, am-

	deum vulgare, Zea mays, and *Sinapis alba.*	monium persulfat, and bromine water.

It will be seen from the above compilation, to which others might be added, that about two dozen methods have been used. Of these mercuric chloride, alcohol, formalin, hydrogen peroxide, or combinations of these have served in the main as the germicide.

The writer tried various of the above methods and finding them unsatisfactory resorted to the use of other substances among which was bleaching powder (calcium hypochlorite). The favorable results which have been secured with bleaching powder and the many requests for the method have prompted the author to present this brief description. The method is simple: Ten grams of commercial chloride of lime (titrating 28 percent chlorine) is mixed with 140 cc. of water. The mixture is then allowed to settle for five or ten minutes and the supernatant liquid decanted off or filtered. The solution or filtrate which contains about 2 percent chlorine is used as the disinfectant. Dilutions from this known strength may be used as well as the full strength. The volume of solution employed should be about five times or more the volume of the seed.

In order to find the exposure at which the ability for germination is affected seeds were placed in sterile test tubes and covered with a one percent chlorine solution, obtained as above, for different lengths of time. Germination tests were then made with the seed which were removed at these different times and compared with untreated seed germinated in the same way.

Seeds were also removed from the disinfecting solution at various intervals and tests made with respect to freedom from bacteria or fungus organisms. The most trustworthy tests were made by planting the seed on the surface of peptone agar and into bouillon. Tubes thus prepared were kept at room temperature, at 30° C., and 37° C. Observations were made on these tubes at intervals up to four weeks. In addition to these tests of sterility many plants which were germinated on agar were used in experimental work where sterility was required and was apparently maintained for several months.

In making transfers the seed was removed from the disinfecting solution by means of a small hand-wrought spoon, momentarily drained and sown in the culture vessel. No attempt was made to remove completely the disinfectant from the seed since it does not seem to interfere with the germination unless the period of treatment is exceedingly long or a noticeable amount is carried over with the seed. In making the transfers, however, an effort was made to leave behind as much of the germicide as possible. That the quantity of disinfecting material carried over with the seed into the bouillon was not sufficient to act as a germicide was proven by placing along with some of the treated seed in the bouillon an untreated seed. In every case within three days there was ample bacterial growth.

With large seeds such as corn, beans, peas, and wheat, only those seeds were used which appeared normal and seemed capable of producing vigorous plants. With other seed, such as timothy, alfalfa, clover, etc., no such selection was made. The number of seeds placed in each series of six to ten tubes varied from one to three with corn and from four to one hundred with other seeds. The number, however, varied with the size of the seed and the amount of available agar surface in the test tube. In several cases as many as 4,000 timothy seeds were treated at a time and transferred to a single container.

The following table presents some of the data.

Seed	Time Required for Sterilizing Seed. Hours	Time Required to Cause Injury to Seed. Hours
Zea Mays L.	8	18
Medicago sativa L.	6	18
Triticum vulgare Vill.	15	20–22
Phleum pratense L.[2]	8	above 22
Brassica Rapa L.[3]	4	" 11
Pastinaca sativa L.[3]	9	" 11
Petroselinum sativum Hoffm.[3]	2 : 15	" 11
Viola tricolor L.[3]	1	" 11
Cosmos bipinnatus Cav.[3]	5	" 10
Brassica oleracea L.[3]	2	" 11
Trifolium pratense L.[3]	24	" 24
Linum usitatissimum L.[3]	10	" 14
Fagopyrum esculentum Moench.[3]	No success at all	" 40
Avena sativa L.[2]	7 : 30	" 15
Solanum tuberosum L.[4]	No success at all	

[2] Seeds hulled before treatment.

[3] Tested only on peptone agar and at room temperature.

[4] Cuttings of tuber.

The data show that the time required in most cases for sterilizing seed and the exposure necessary to produce injury are many hours apart. This allows considerable latitude with respect to the time during which the disinfectant may be allowed to act. It is evident also that the different seeds are not sterilized in the same length of time, alfalfa requiring about six hours while wheat requires more than twice as long.

Other tests of the efficiency of the method have been made. In one experiment thirty or more carefully selected seeds of each of the different kinds used were placed in test tubes and covered with a calcium hypochlorite solution containing approximately two percent chlorine. After treatment the seeds were transferred into test tubes on the surface of a medium which contained tap water and one percent each of agar and saccharose. After germination of the seed each tube was inoculated with a pure culture of *Bacillus radicicola* and placed in the greenhouse where it remained 45 days before examination. The seeds used and the time of treatment of each are as follows:

Seed	Time of Treatment
Medicago sativa L.	7 hours
" *hispida* Gaertn.	7
" *media* ?	7
" *falcata* L.	7
" *lupulina* L.	7
Melilotus alba Desr.	7
" *indicata* (L.) All.	7
" *officinalis* (L.) Lam.	7
Vicia villosa Roth.	6½
" *sativa* L.	6½
" *Faba* L.	3
Lathyrus sylvestris L.	5
" *sativus* L.	4

The above treated seeds were planted in 184 test tubes which were reopened when inoculated. On examination only three tubes showed signs of contamination. Two of these were contaminated with molds while the other was with a bacterial organism.

In addition to the foregoing tests the following data on the efficiency of the method were contributed by Dr. Knudson from experiments made on the organic nutrition of plants. These data are valuable because they indicate the thorough sterilization of the seed as is shown by the freedom of cultures from contaminations even after 30 days or more of culture. The duration of immersion of the seed in the disinfectant and the culture media employed are indicated in the following table.

Seed	Immersion Hours	Media. Pfeffer's Solution ½ Strength to which was Added in Separate Lots
Phleum pratense L.	7½	Saccharose, glucose, levulose, maltose and lactose each 2%.
Zea Mays L.	10	Glucose, levulose, maltose and saccharose 2%. One series in the laboratory and one in the greenhouse.
Vicia villosa Roth. (100 seeds)	6	Lactose, maltose, saccharose, 1% each. Sterilization not always successful.
Raphanus sativus L.	6	Lactose, maltose, dextrose, and saccharose, each 1%.
Brassica oleracea L.	11	One series in greenhouse and one in laboratory on saccharose, maltose, levulose, lactose, each in four concentrations from one tenth percent to 2%.
Linum usitatissimum L.	11	Maltose and lactose each 1%.
Pisum sativum L.	4½	Saccharose, lactose, maltose, glucose each 1% and checks.

Satisfactory sterilization was secured in every case with the exception of the vetch seed. In certain experiments where only a few vetch seeds were employed no contaminations occurred in the cultures except occasionally where it was clearly evident that contamination was due to causes other than the failure of the hypochlorite. In certain experiments on the influence of sugars on respiration, 100 seeds were required for each culture chamber. Failure to sterilize occurred in 20 out of 40 trials and in each case it was due to introduction of one or more dead seed along with others.

The considerable number of experiments made in which this method of seed sterilization has been employed and in which sterile plantlets have been secured and maintained over a period of thirty days or more conclusively demonstrate the efficiency of the method as an aid for securing sterile plantlets. The ease of operation and the fact that the solution does not injure the seed except after long exposure to the hypochlorite solution make the method particularly desirable. The effect of the solution is probably due to the hypochlorous acid, as suggested by Hooker, which acts as the toxic agent. In conclusion it should be added that the method not only offers a way for securing sterile plantlets for physiological experiment but also for eradicating such plant diseases as may be controlled by treating the seed.

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SEASONAL DURATION OF ASCOSPORE EXPULSION OF ENDOTHIA PARASITICA*

F. D. HEALD AND R. A. STUDHALTER

The effect of moisture upon the expulsion of ascospores of the chestnut blight fungus, *Endothia parasitica* (Murr.) And., was first studied by Rankin (1). Later the same writer (2) made the following report: "The speaker found the ascospores being shot from mature pustules during every rainy period last summer" (1911). A more detailed study of ascospore expulsion was made during the following summer by Anderson and Babcock (3). The main conclusion of these writers on the moisture relation can best be presented in their own words: "We may say then in general that, as long after a rain as the surface of the bark remains wet, the spores will continue to shoot."

That certain temperature conditions must be fulfilled to insure spore expulsion was first brought out by laboratory tests reported by the senior author (4). Later in a discussion of "The relative prevalence of pycnospores and ascospores of the chestnut blight fungus during the winter," Heald and Gardner (5) report the results of field tests made during the winter of 1912–13, showing that ascospores were not expelled during the warm winter rains. A brief statement of the relation of temperature to the expulsion of ascospores of *Endothia parasitica* has been published by Walton (6) and a more detailed consideration by Heald and Walton (7) of the temperature relation based on laboratory tests in constant temperature rooms has substantiated the earlier results. The only previous work bearing on the seasonal duration of ascospore expulsion is that of Rankin to

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* During the first part of the period covered by this paper, Mr. M. W. Gardner was associated with the writers, and he deserves special mention for careful and painstaking work in connection with the records and observations made at West Chester.

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which reference has been made, and observations made by Anderson and Babcock (3) during every rain period for the month of August, 1912.

It seemed desirable to have continuous records of the behavior of definite groups of perithecial pustules through one or more seasons under natural field conditions. For this purpose the work presented in the following pages was undertaken.

Method and Plan

In carrying out this work the first requisite was a means of holding a glass slide over a definite area of perithecia-bearing bark. For this purpose a wooden bracket was devised. This is a small rectangular block, the width of an object slide, carrying a slot at one end for the insertion of the slide, and attached to the tree by a brass screw passing through the center (Fig. 1). On some trees the bracket was directed downwards, in which case the object slide was held in position by a screw turned into the bark (Fig. 1, *A*). In both cases the object slide was always wedged firmly into the slot. Ascospore

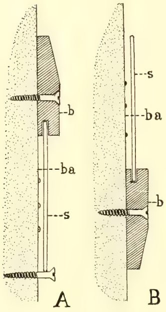

FIG. 1.

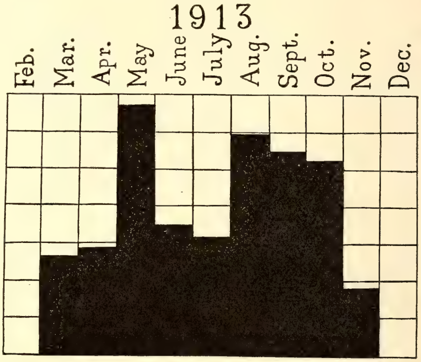

FIG. 2.

FIG. 1. Diagram of ascospore traps, showing the two positions employed; *b*, bracket; *ba*, bark bearing perithecial pustules; *s*, object slide for catching the expelled spores.

FIG. 2. Diagram showing the relative intensity of ascospore expulsion at West Chester, Pa., during the season of 1913.

expulsion was recorded equally well by slides in either position. Form *A* was designed to prevent excessive washing of the slide by the rain, while form *B* allowed a more ready penetration of the water. This device has been designated as an "ascospore trap" to distinguish it from other kinds of spore-traps (5, 8).

The original plan called for the placing of sets of ascospore traps on typical lesions under three different field conditions: first, in young chestnut coppice; second, in a chestnut orchard; third, in a forest. Owing to lack of assistance it was necessary to omit the traps from forest trees. A suitable chestnut coppice was found near West Chester, Pa., and the orchard of the Paragon Fruit and Nut Co., Martic Forge, Pa., was selected for the second set of traps. A set of standard weather instruments, including a rain gauge, Friez thermograph, and Friez hygrograph was installed at each station. Following each rain or rain period the slides were collected and brought to the laboratory and a record made of the amount of ascospore expulsion shown for each trap.

The Traps at West Chester

A total of 76 ascospore traps were set on trees 4 to 8 inches in diameter, in a badly diseased coppice growth of native chestnut. Trees were selected so as to give varying conditions of the lesions, with perithecia in different stages of development. Some of the pustules showed perithecia which were just reaching maturity when the traps were set while others judging from the conditions of the pustules had probably expelled spores during the previous summer. The first traps (1–16) were set on November 26, 1912; a second lot (17–32) were placed December 3, 1912; another addition (33–36) was made December 18, 1912; and the number was further increased (37–49) on December 31, 1912. Nos. 1–49 inclusive were under continuous observation during all or part of the winter of 1912–13. During the spring several more traps (50–52) were set. A fence made of chestnut rails gave an opportunity to test ascospore expulsion under different conditions, and on May 1, 1913, traps (53–56) were placed over areas of perithecial pustules on several rails. The last traps (57–76) were set August 15, 1913. Nos. 50–76 included no perithecia that had reached maturity and expelled spores during the previous season (1912).

The Results from the Traps at West Chester

From November 26, 1912 to March 19, 1913, there were 19 rain periods with precipitation varying from 0.01 to 1.8 inches, and maximum temperatures from 37° to 64° F. during the days of the rain, with no expulsion of ascospores (Table I). The first expulsion of ascospores for the season of 1913 occurred on March 20 following a rainfall of 1.64 inches and expulsion continued through the following months, but was checked by unfavorable temperatures early in November (Table II). From November 16, 1913 to May 4, 1914, there were 18 rain periods with precipitation varying from 0.10 to 1.95 inches and maximum temperatures from 34°–68° F. during the days of the rain, and no expulsion of spores. The first expulsion of ascospores for the season of 1914 occurred on May 5, following a rain of 0.71 inches (Table III). It may be noted that the first expulsion for 1914 was about six weeks later than in the spring of 1913. The records were continued to the end of July, 1914 (Table IV), when there was a forced discontinuance of the work.

Table I

Ascospore Trap Tests, Records for West Chester, Pa., Winter and Spring, 1912–13

Rainfall		Temperature		Percentage of Traps Showing Expulsion
Date	Amount, Inches	Max.	Min.	
Nov. 28	0.01	38	30	0
Dec. 2	0.36	56	38	0
“ 4–6	0.5	68	36	traces
“ 11	0.03	43	37	0
“ 17–18	0.2	46	30	0
“ 19	0.89	53	33	0
“ 24	9.5 (snow)	34	25	0
“ 27	1.02	37	32	0
“ 30	1.8	46	36	0
Jan. 3	0.93	58	37	0
“ 4	0.1 (snow)	39	27	0
“ 7–8	0.78	60	36	0
“ 10	0.13	40	22	0
“ 11–12	0.24	59	32	0
“ 15–16	0.81	46	26	0
“ 17–18	0.31	57	33	0
“ 20	0.24	54	33	0
“ 23–27	0.88	57	28	0
Feb. 12	0.15	35	15	0
“ 22	0.07	58	45	0
“ 27	0.78	60	38	0
Mar. 10	0.53	49	38	0
“ 13–15	1.375	64	29	0
“ 20	1.64	63	49	47

TABLE II

Ascospore Trap Tests, Records for West Chester, Pa., 1913

Rainfall		Temperature		Percentage of Traps Showing Expulsion
Date	Amount, Inches	Max.	Min.	
Mar. 20	1.64	63	49	47
" 26–27	1.18	69	38	47
Apr. 4	0.56	76	44	21.5
" 10–13	2.49	58	27	36.5
" 13–16	1.57	67	41	4
" 27–28	2.43	74	48	65.4
" 29	0.11	56	54	8
May 16–17	0.65	80	48	46.4
" 21–24	3.24	77	46	70.3
" 27–28	0.69	72	53	83.3
June 7	0.12	83	64	0
" 20	0.08	88	62	50
" 21	0.02	82	65	11.1
" 24	0.19	78	65	52
" 27–28	0.22	86	66	66
July 5–6	0.16	92	69	28.8
" 9	0.30	75	56	47.1
" 12	0.75	86	56	88.5
" 17–18	0.20	87	57	27.5
" 21	0.05	82	61	4
" 24	0.04	88	66	4
" 28	0.06	91	70	0
" 29–30	0.74	96	68	60.8
Aug. 1	0.05	83	72	11.5
" 6	1.98	83	62	74
" 10	0.37	93	72	75.5
" 18	0.03	93	70	0
" 19	0.40	80	62	67.6
" 22	0.25	76	50	63.4
" 27	0.175	85	63	50.7
" 29–30	1.10	81	62	74.6
Sept. 7	0.37	86.5	66.5	62.8
" 13	0.095	70	58	18.5
" 17–18	0.26	74.5	52	57.7
" 18–19	0.68	74.5	58	62
" 19–20	0.09	65.5	57	34.2
" 20–22	1.26	79	48	77.1
Oct. 1–3	1.30	71	52	54.3
" 11	0.73	69.5	62	67.1
" 19–20	0.86	69	50	64.3
" 20	0.08	69	54	2.8
" 24–26	1.44	64	49	74.3
Nov. 8–9	1.00	60	37	54.3

The percentage of traps showing expulsion for any rain period during the season of perithecial activity varied from 0 to 88.5 (Tables I–IV). The failure to get expulsion of spores from all traps depended on the following conditions:

1. Temperature below the optimum for expulsion.
2. Insufficient rainfall.
3. Location of the trap with reference to the direction of the rain.
4. Condition of the perithecia (not in proper stage to expel spores).

TABLE III

Ascospore Trap Tests, Records for West Chester, Pa., Winter and Spring, 1913–14

Rainfall		Temperature		Percentage of Traps Showing Expulsion
Date	Amount, Inches	Max.	Min.	
Nov. 8–9	1.00	60	37	54.3
" 15–16	0.96	58	28	0
" 28	0.62	44.5	33.5	0
Dec. 7	0.53	52	41	0
" 21	0.05	47	27	0
" 23–5	1.95	50	32	0
Jan. 3–5		39	25	0
" 24	1.35	50	40	0
" 31	0.88	63	36	0
Feb. 6–7	0.62	48	27	0
" 13–14	4.5 (snow)	32	0	0
" 16	1.5 "	25	2	0
" 19	0.68	34	29	0
Mar. 1–2	4.50 (snow)	38	15	0
" 5–6	6.00 "	33	29	0
" 11	traces "	29	20	0
" 18	0.10	43	29	0
" 20–22	3.5 (snow)	34	11	0
" 28	0.35	58	37	0
April 1	0.25	48	37	0
" 8	0.60	67	41	0
" 15	0.49	45	41	0
" 20	0.44	68	45	0
" 25	1.57	50	44	0
" 30	0.10	50	45	0
May 5	0.71	65	59	11.8

There was a marked example of the influence of the temperature following a rain of 1.57 inches on April 13–16, 1913, when only 4 percent of the traps showed expulsion. The maximum rainfall following which there was no expulsion of ascospores when temperature conditions were favorable was 0.12 inches on June 7, 1913. A small percentage of traps sometimes showed expulsion when the rainfall was less than 0.12 inches. (See Tables I–IV.) The number of rains for the entire period covered by our investigations following which there was expulsion of ascospores is shown in Table V.

Perithecial activity in 1913 began in the spring as soon as temperature conditions became favorable, but since the temperatures at this

TABLE IV

Ascospore Trap Tests, Records for West Chester, Pa., Spring and Summer, 1914

Rainfall		Temperature		Percentage of Traps Showing Expulsion
Date	Amount, Inches	Max.	Min.	
May 5	0.71	65	59	11.8
" 8	0.21	58	52	traces
" 12	0.56	72	45	"
" 27	0.19	92	65	3
June 4	0.30	70	61.5	9.2
" 14–15	0.08	75.5	58	0
" 21–22	1.08	80	48	26.1
" 27	0.68	86	60	29.2
July 2	1.15	82	64	24.6
" 5	0.35	73	61	34.3
" 7	0.70	70	59	31.1
" 13–15	3.14	85	66	36.6
" 16	0.25	83	69	40
" 25	0.31			16.6
" 28	3.58			33.3

TABLE V

Summary of Number of Rain Periods and Number of Times Spores Were Expelled

Period	No. of Rain Periods	Amount of Rain in Inches	No. of Rains During Which Expulsion Occurred
Nov. 26, 1912–Mar. 19, 1913	19	12.125	0
Mar. 20, 1913–Nov. 15, 1913	42	30.01	39
Nov. 16, 1913–May 4, 1914	18	11.64	0
May 5, 1914–July 31, 1914	15	13.04	14

TABLE VI

Records for Ascospore Traps, Martic Forge, Pa., Winter of 1912–13

Rainfall		Temperature		Percentage of Traps Showing Expulsion
Date	Amount, Inches	Max.	Min.	
Jan. 29	.08	38	31	0
" 31	.06	62	42	0
Feb. 4	.70	37	28	0
" 10	.03	32	18	0
" 17	.04	35	29	0
" 22	.09	56	49	0
" 27	.36	58	38	0
Mar. 5	.02	45	31	0
" 10	.55	52	41	0
" 13	.10	55	53	0
" 14	1.29	67	53	2

time were generally below the optimum for spore expulsion, the highest activity was not reached until the month of May. There was a decline in spore expulsion during the months of June and July, but there was abundant expulsion in August, September and October. In November, with the return of unfavorable temperatures, the perithecia ceased to expel spores. This seasonal relation is represented graphically in Fig. 2.

TABLE VII

Records for Ascospore Traps, Martic Forge, Pa., Spring and Summer, 1913

Rainfall		Temperature		Percentage of Traps Showing Expulsion
Date	Amount, Inches	Max.	Min.	
Mar. 14	1.29	67	53	2.0
“ 15	.92	61	56	6.0
“ 20	.62	66	49	6.0
“ 21	.03	68	52	0
“ 26–27	3.47	65	30	68.0
“ 31	.04	69	49	0
Apr. 3	.10	74	39	0
“ 5	.23	59	40	19.6
“ 14	1.78	57	51	65.3
“ 16	1.15	59	45	0
“ 28	2.33	58	45	98.0
“ 29	.07	49	35	4.0
May 15	.11	74	42	2.0
“ 16–17	.74	84	49	98.0
“ 21	.27	64	50	58.0
“ 29–30	.69	75	44	95.0
June 19–21	.34	92	52	88.0
“ 23	.26	75	61	96.0
“ 27	.37	101	77	100.0
July 2 and 5	1.97	97	66	100.0
“ 9	.59	69	63	100.0
“ 17 and 20	.13	89	57	96.0
Aug. 1	2.68	87	68	100.0
“ 6	1.37	82	64	98.0
“ 10	1.13	97	70	98.0
“ 22	.17	73	58	100.0
“ 29	.30	79	68	100.0
Sept. 7	.67	92	69	86.0
“ 12	.09	67	56	68.0
“ 17–18	.19	75	54	82.0
“ 20–21	2.37	80	57	92.0
“ 29	.02	68	55	0
Sept. 30–Oct. 2	1.63	77	50	100.0
Oct. 11	.67	68	56	94.7
“ 19	1.05	57	46	85.0
“ 20	.15	68	42	1.4
“ 25–26	1.04	61	50	93.3
Nov. 8–9	.91	58	33	83.3
“ 15–16	.83	40	25	0

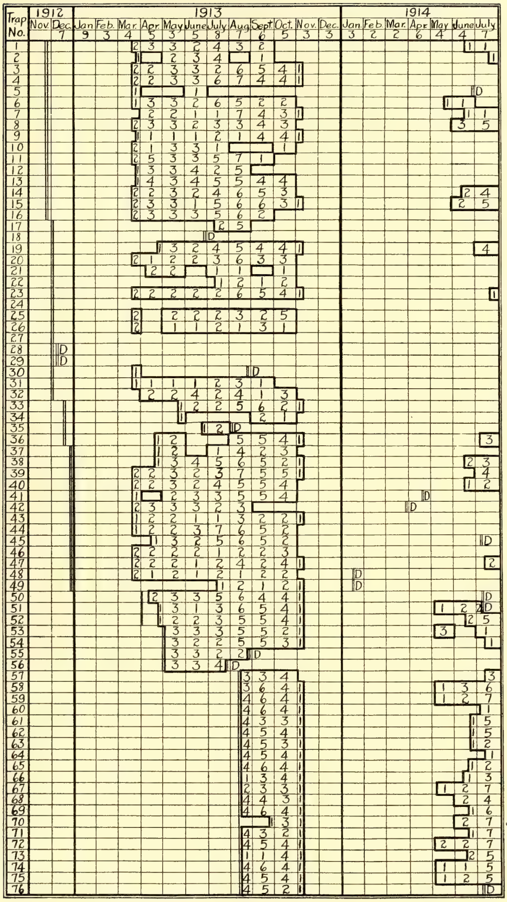

FIG. 3. Chart showing the seasonal duration of ascospore expulsion at West Chester, Pa. The vertical parallel lines indicate the approximate time when the traps were set. D following two parallel lines indicates the discontinuance of a trap. The figures below each month refer to the number of rain periods for the month, while the figures opposite each trap number represent the number of rain periods for the month following which there was an expulsion of ascospores.

At the beginning of our work it was an open question as to whether perithecial pustules would exhaust their power to expel spores during the course of a single season. Our records show that bark bearing stromata with perithecia that probably were active during the season of 1912, gave an abundant expulsion during the season of 1913 (Traps 1–49). For these same traps there was either no expulsion or it was irregular and spasmodic during the season of 1914. Somewhat more certain evidence of the continuation of perithecial activity during two seasons is shown by the records for traps 57–75 from which there was an abundant spore expulsion during the season of 1913 and the part of the season of 1914 covered by our observations. This duration of ascospore expulsion from the different traps and also their seasonal activity is represented in an accompanying chart (Fig. 3).

Records were obtained on the majority of the 76 traps for the entire period covered in this report. Sixteen traps were discontinued before the expiration of the work for various reasons. In 9 cases the bark was shed from the tree due to the action of blight and accessory agencies, in 4 the bark was overgrown by sporophores of sap rot fungi, while one was overrun by a foreign mycelium.

The Traps at Martic Forge

A total of 75 ascospore traps were set in the chestnut orchard at Martic Forge on Paragon trees ranging from 3 to 12 inches in diameter. The pustules over which the traps were placed were in varying stages of development, but were on the whole much younger than those at West Chester.

The first traps (1–41) were set on January 21–24, 1913; four (42–45) were added on January 31; three (46–48) on February 10; and two more (49–50) on February 12. An addition of twenty-five traps (51–75) was made early in October 1913, and the first record on these was taken for the rain of October 11. These last 25 traps were placed over pustules which had just reached sufficient maturity to expel spores during the season of 1913.

The Results from the Traps at Martic Forge

There were 10 rain periods between January 29 and March 13, 1913, with a precipitation ranging from 0.02 to 0.70 inches (Table VI). No expulsion of ascospores occurred during this period. Temperatures favorable for perithecial activity were first offered on March 14, 1913, when 2 percent of the traps shot spores following a rain of 1.29 inches. From March 14 to November 9 there were 38 rain periods with precipitation varying from 0.02 to 3.47 inches (Table VII). During most of these rain periods there was abundant expulsion of ascospores, and following seven of them all of the traps expelled spores. After November 9 the temperatures were again unfavorable for perithecial activity, and no expulsion occurred for any of the 21 rains between November 10, 1913 and April 15, 1914 (Table VIII). Beginning with April 20, with the return of higher temperatures, there was again an abundance of expulsion following every rain period until July 31, 1914, when the work was discontinued (Table IX).

Table VIII

Records for Ascospore Traps, Martic Forge, Pa., Fall and Winter, 1913–14

Rainfall		Temperature		Percentage of Traps Showing Expulsion
Date	Amount, Inches	Max.	Min.	
Nov. 8–9	.91	58	33	83.3
" 15–16	.83	40	25	0
" 27–29	.54	49	32	0
Dec. 1	.06	44	33	0
" 7	.51	52	37	0
" 21	.13	49	26	0
" 23–25	2.25	49	28	0
Jan. 3–5	2.11	41	25	0
" 24	1.21	47	39	0
" 31	.83	61	34	0
Feb. 6–7	.48	46	24	0
" 13–14	1.12	30	3(?)	0
" 19	.62	31	26	0
Feb. 28–Mar. 1	.38	40	18	0
Mar. 5–6	.72	36	17	0
" 11	.03	30	18	0
" 20–22	.34	37	17	0
" 28	.36	59	36	0
Apr. 1	.28	45	35	0
" 8	.59	65	35	0
" 11	.07	59	33	0
" 15	.70	45	39	0
" 20	.59	67	53	10.8

It will be noted (Tables I and VI) that in the spring of 1913 expulsion began at Martic Forge a few days earlier than at West Chester. In the fall of 1913 perithecial activity ceased with the same rain period at both places, but in the following spring the Martic Forge traps were again the first to expel spores. There was very little difference between the temperatures at the two stations. The earlier start at Martic Forge is accounted for by the fact that the pustules over which the traps were set were on the whole much younger and in better condition to expel spores than those at West Chester. During the seasons of perithecial activity for both 1913 and 1914, a larger percentage of the traps at Martic Forge expelled spores than at West Chester. Furthermore a much more copious expulsion was obtained from individual traps at Martic Forge.

TABLE IX

Records for Ascospore Traps, Martic Forge, Pa., Spring and Summer, 1914

Rainfall		Temperature		Percentage of Traps Showing Expulsion
Date	Amount, Inches	Max.	Min.	
Apr. 20	.59	67	53	10.8
" 25–26	1.57	68	44	2.7
May 5	1.11	66	60	27.0
" 8	.13	62	50	32.9
" 12	.40	81	47	16.2
" 28	.42	87	60	5.6
June 4	.42	72	64	46.6
" 8	.22	97	65	20.8
" 18 and 21–23	.97	91	50	43.1
" 23–25 and 28	2.10	94	62	68.6
July 2–3	1.63	80	54	55.6
" 4–5	.28	73	57	78.1
" 10 and 12	.95	90	66	69.2
" 13–14	.23	84	65	69.9
" 14–15	.53	86	66	75.3
" 15–16	2.31	86	66	53.4
" 24–25	1.17	91	67	45.8
" 28	2.61	81	55	56.2

TABLE X

Summary of the Number of Rain Periods and Number of Times Spores were Expelled at Martic Forge, Pa.

Period	No. of Rain Periods	Amount of Rain in Inches	No. of Rains During which Expulsion Occurred
Jan. 29, 1913–Mar. 13, 1913	10	2.03	0
Mar. 14, 1913–Nov. 9, 1913	38	31.94	33
Nov. 10, 1913–Apr. 19, 1914	23	14.16	0
Apr. 20, 1914–July 31, 1914	18	17.64	18

Under laboratory conditions the minimum temperature for ascospore expulsion was found to be between 50 and 55° F. While it was impossible to get the maximum and minimum temperatures for the exact period during which ascospores were expelled after each rain, still the maximum and minimum temperatures during each rain period (Tables VI–X) show that the minimum temperature for expulsion in the field corresponded quite closely with that in the laboratory. The rain of April 14, 1913, offers a good example of a heavy rain with no expulsion, due to low temperatures (Table VII). Although copious expulsion occurred both before and after this date, and the precipitation had been heavy (1.15 in.), the temperatures were below the minimum for perithecial activity.

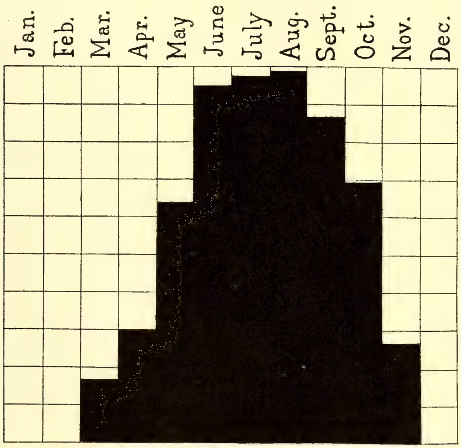

FIG. 4. Diagram showing the relative intensity of ascospore expulsion at Martic Forge, Pa., during the season of 1913.

The number of rains for the entire period covered by our investigations and the number of rains following which ascospore expulsion took place, are shown in Table X.

The seasonal relation of expulsion at Martic Forge for the year 1913 was somewhat different from that at West Chester. At the latter place the monthly percentage of traps showing expulsion increased suddenly from March to a maximum in May; during June and July there was a decided decrease, but after July there was abundant expulsion until November (Fig. 2). At Martic Forge there

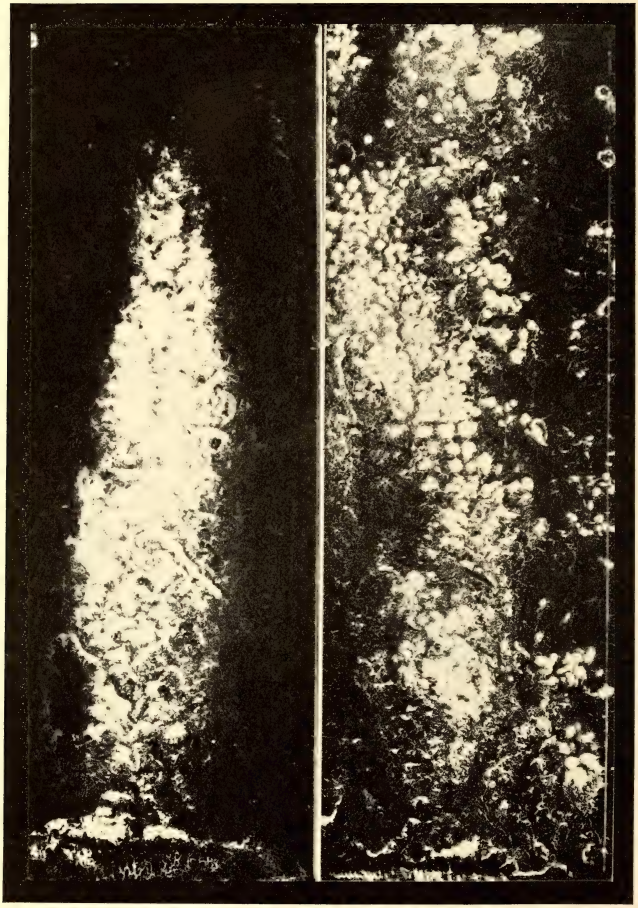

FIG. 5. Photographs of slides from trap 52 (upper) and trap 62 (lower). Slide from 52 was taken following a rain of 1.63 inches on July 2–3, 1914; slide 62 following a rain of 0.95 inches on July 10–12, 1914.

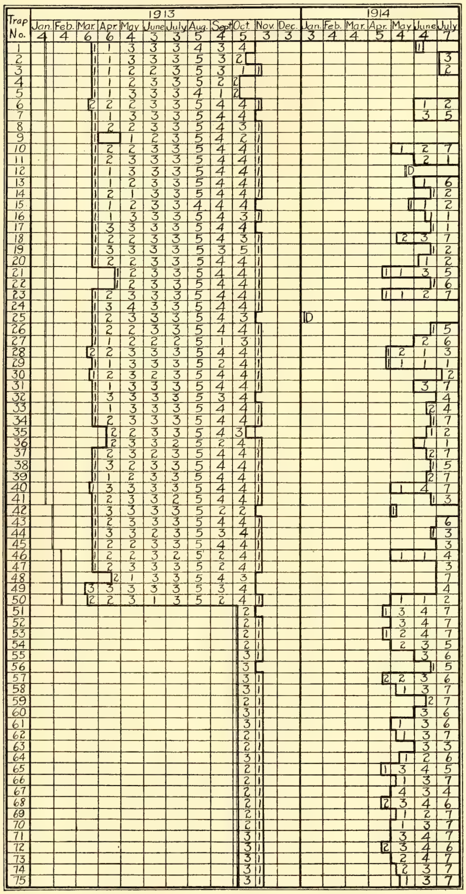

FIG. 6. Chart showing the seasonal duration of ascospore expulsion at Martic Forge, Pa. (See legend of Fig. 3 for more detailed explanation.)

was a gradual increase from the beginning of expulsion in March to a maximum in August, followed by a more sudden, though steady, decrease until November (Fig. 4). The maximum at Martic Forge (98.8 percent) was also much higher than that at West Chester (67.3 percent). It is difficult to explain this seasonal difference except to suppose that it might have been due to the difference already mentioned in the condition of the pustules.

TABLE XI

Record of Expulsion from Typical Young and Mature Pustules at Martic Forge for the Active Season, 1913

Rainfall		Trap Numbers and No. of Active Ostioles							
Date	Amount, Inches	Young Pustules				Mature Pustules			
		21	23	37	40	11	17	28	43
Mar. 15	.92	0	0	0	0	0	0	6	0
" 20	.62	0	0	0	10	0	0	0	0
" 26–27	3.47	0	N	14	0	15	8	N	28
Apr. 3	.10	0	0	0	0	0	0	0	0
" 5	.23	0	0	0	27	0	1	0	0
" 14	1.78	0	N	P	P	31	N	1	27
" 16	1.15	0	0	0	0	0	0	0	0
" 28	2.33	2	N	N	19	N	N	100	N
May 15	.11	0	0	1	0	0	0	0	0
" 16–17	.74	25	N	N	N	N	N	N	N
" 21	.27	0	1	0	1	14	16	21	N
" 29–30	.69	N	N	64	N	N	N	N	11
June 19–21	.34	P	P	0	95	N	N	1	7
" 23	.26	N	N	6	N	N	N	N	N
" 27	.37	N	13	N	N	N	N	N	N
July 2 and 5	1.97	100	58	100	N	N	N	N	73
" 9	.59	N	N	100	N	70	50	N	50
" 17 and 20	.13	18	4	1	5	2	41	6	2
Aug. 1	2.68	N	N	47	N	48	P	P	54
" 6	1.37	N	32	38	36	22	18	8	65
" 10	1.13	35	N	N	40	26	24	18	27
" 22	.17	22	66	4	33	34	22	11	21
" 29	.30	N	43	4	49	37	27	15	20
Sept. 7	.67	N	N	29	37	10	5	24	N
" 12	.09	N	18	5	19	4	P	P	5
" 17–18	.19	N	N	3	27	23	P	7	P
" 20–21	2.37	N	N	N	N	17	P	5	33
" 30–Oct. 2	1.63	N	N	N	N	14	6	P	34
Oct. 11	.67	N	N	N	N	17	5	11	26
" 19	1.05	N	N	N	26	13	27	3	5
" 20	.15	0	0	0	0	0	0	0	0
" 25–26	1.04	N	N	N	N	15	2	9	8
Nov. 8–9	.91	N	65	N	N	19	0	10	8
" 15–16	.83	0	0	0	0	0	0	0	0

N = numerous.

The fact that perithecial pustules would not exhaust their power to expel spores in the course of a single season is even more clearly shown by the traps at Martic Forge than by those at West Chester. Pustules tested by traps 1–50 must have expelled spores during 1912, before the traps were set. All of these traps showed active ostioles during 1913, and all but five of them expelled some spores during 1914. Pustules tested by traps 51–75 probably expelled their first spores during the spring and summer of 1913. All of these showed perithecial activity during 1914, most of them expelling spores in large numbers. Fig. 5 represents slides taken from traps 52 and 62 during July, 1914, and shows how copious the expulsion may be during the second season. No heavier shooting was obtained from any of the traps even during their first season of activity.

The accompanying chart (Fig. 6) represents the duration of expulsion and the seasonal activity for the traps at Martic Forge.

In some instances the tree was entirely dead and the bark peeling off at the time the trap was set, early in 1913. Nearly all of these cases showed continued perithecial activity during 1913, and most of them had not entirely lost their power to eject spores in 1914. This is best shown by traps 10, 43 and 50 (Fig. 6).

There is a difference between the continued activity of young and mature pustules. The former continue to shoot spores unabated throughout the entire season, whereas the maturer pustules eject a larger number of spores during the early part of the season and show a gradual decline toward the winter months. In Table XI is presented the record of expulsion of ascospores for each rain period during the active season of 1913 for typical young and mature pustules.

Expulsion of Ascospores from Bark Shed from Old Infections

Trap 41 at West Chester was discontinued in April, 1914, the bark to which the trap was attached being shed from the tree. In order to determine whether perithecia in bark shed from trees would continue to expel spores, five representative pieces from bark collected on the ground beneath trap 41 were brought to the laboratory and tested for ascospore expulsion. Each of the pieces (1 × 3 inches) showed active ostioles, the maximum number for any day during the progress of the test being forty. This activity was less than from similar bark taken directly from cankers. Bark shed from the rail fence adjacent to traps 53 and 54 was also tested for spore expulsion with positive results. These results give added emphasis to the statement already made (7) that the chestnut blight fungus has a most remarkable power of ascospore production.

Discussion

A comparison of the results obtained at the two stations shows a very general agreement in the more important features. All of the traps at West Chester were set on native coppice while those at Martic Forge were on Paragon trees grafted on native coppice. Some of the traps at the latter station were set below the graft unions. The greater activity of the perithecia during the second season at Martic Forge as compared with the results at West Chester is probably due to the host relation, permitting a somewhat more rapid maturing of the fungus at the latter place.

Since the prolonged perithecial activity established by our observations for *Endothia parasitica*, is not in accord with what is known in regard to many other pyrenomycetous fungi, an explanation for this peculiarity should be sought. There seem to be two important features in the development of the fungus that contribute to prolonged perithecial activity. First, the period of successive maturing of asci is very extended. This fact is substantiated by the work of Heald and Walton (7) on the expulsion of ascospores under laboratory conditions, and is in agreement with the study by Anderson (9) of the morphology of the perithecia. The second feature not so clearly revealed by the morphological studies (9) appears to be the successive maturity of the perithecia in a given stroma. All perithecia in a given stroma do not reach maturity at the same time, some being still buried deep in the stromata when the necks of others adjacent have reached the surface and are in a condition to expel spores. Add to the above features the fact that there is no seasonal relation to the maturing of perithecia and one must admit that *Endothia parasitica* is remarkably well equipped for the continuous production of ascospores.

During the progress of our work detailed examinations were made of the perithecial pustules included under each trap, their condition being recorded at the beginning of the tests and at intervals thereafter. As a result it can be definitely stated that the development of new stromata was the exception, the bulk of all expulsion recorded coming from stromata that were present at the beginning of the tests.

SUMMARY

1. Expulsion of spores from the perithecia of *Endothia parasitica* begins in the spring with the first warm rains, and increases to a maximum of activity as conditions become more favorable, to be followed by a decline in the fall when lower temperatures prevail, and ceases entirely during the cooler portions of the year, although there may be an abundant rainfall. During 1913 the period of expulsion extended from about the middle of March to the middle of November. For 1912–13 there were 19 rain periods during the fall, winter and spring with no expulsion of ascospores, and in 1913–14 there were 18 rains which induced no expulsion of ascospores. During one third to one half of the year there is then no expulsion of ascospores, but during the remainder there is an abundant expulsion with each rain of any consequence (0.10+) except in a few instances when the temperature drops below the minimum.

2. Perithecial pustules of the chestnut blight fungus show a remarkable power of spore production. This power is not exhausted during the course of a single season, and in many cases is as marked during the second season as during the first. Our results point also to the conclusion that pustules first producing mature perithecia in the fall may continue to be a source of spores during the two following seasons of perithecial activity, the maximum of production occurring during the season following their maturity, with a gradually diminishing production of spores during the next season. This remarkable power is due to successive maturity of asci, to successive maturing of perithecia, and the successive maturing of stromata throughout the season.

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4. **Heald, F. D.** Pathological Investigations. Rep. Pa. Chestnut Bl. Comm., July 1 to Dec. 31, 1912: 40–42, figs. 22–38. 1913.
5. **Heald, F. D. and Gardner, M. W.** The Relative Prevalence of Pycnospores and Ascospores of the Chestnut Blight Fungus During the Winter. Phytopathology **3**: 296–305, *pls. 26–28*. 1913.

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DIMORPHISM IN CONIOTHYRIUM PIRINUM SHELDON[1]

C. H. Crabill

Several years ago the writer began a study of the Phyllostictas and Coniothyriums associated with the frog-eye leaf spot of apple. In a previous paper[2] the morphology, cultural features, and host relationships of *Coniothyrium pirinum* were briefly dealt with. The following report is the outcome of pure culture studies of *C. pirinum* isolated from apple leaf spots.

The first cultures of this fungus, Strain I, were secured in 1911 from Black Ben Davis leaves at Blacksburg, Va. In 1913 another strain, Strain II, was obtained from the same source. Although the mycelium, pycnidia and spores of the two are in no wise to be distinguished from each other under the microscope, the two strains are readily distinguished when grown in Petri dish culture on artificial media. Starch agar[3] has been used almost exclusively in these studies. Plates of this agar were poured, cooled, and inoculated at the center by the use of a platinum wire. Tubes of this medium were used in making dilution and stock cultures.

Strain I, four years after isolation, exhibits the same characters as when first secured. It may be described as follows:

PETRI DISH CULTURE:[4] STARCH AGAR

Mycelium pure white or pinkish until about the 10th day, acquiring then in spots, which spread gradually throughout, an olivaceous color. Pycnidia very few, large, multilocular, prolific, irregularly distributed about the point of inoculation, appearing 8 to 30 days after inoculation (Fig. 1).

Strain II when first isolated was described as follows:

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[1] Paper No. 40 from the laboratories of Plant Pathology and Bacteriology, Va. Agr. Exp. Sta.

[2] Crabill, C. H. Studies on Phyllosticta and Coniothyrium occurring on apple foliage. Rep. Va. Agr. Exp. Sta. 95–115. 1911–12.

[3] Made according to the following formula: H_2O, 900 cc.; $MgSO_4$, .5 g.; K_2HPO_4, 1.0; $NaNO_3$, 2.0; KCl, .5; $FeSO_4$, .1; Agar agar, 20.0. Cook, filter, and while still hot add the following paste: H_2O (cold), 100 cc.; cornstarch, 10 g.; and sterilize.

[4] Unless otherwise stated, cultures were incubated in moist chamber.

PETRI DISH CULTURE: STARCH AGAR

Mycelium pure white or pinkish until about the 5th day, when an olivaceous color begins to develop just inside the margin of the colony. Pycnidia multitudinous, small, prolific, uniformly distributed over the colony, appearing 3 to 6 days after inoculation (Fig. 2). As the pycnidia develop, the olivaceous color is absorbed, so that the greenish zone near the margin of the colony is continually advancing.

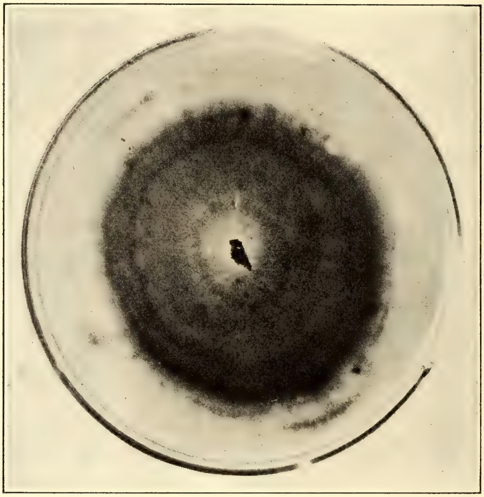

FIG. 1. Strain I in about the 43d generation, 24 days old. The tardy production of relatively few large multilocular pycnidia is characteristic of this strain. A few young pycnidia have just appeared in this culture.

Note: The photographed plates here figured may be located on the chart shown in Fig. 6 by sub-numbers which correspond to the numbers of the figures.

For a year after isolation, Strain II was grown in test-tube cultures and transferred to fresh media every two months. At the end of that time plates of starch agar were center inoculated with it. Mycelium, as well as spores, was transferred. All of the colonies which developed showed fruiting and non-fruiting sectors. The fruiting sectors were in all respects typical of Strain II. The non-fruiting sectors produced typical mycelium which however never developed either color or fruiting bodies, although they were kept for two months.

Other plates were then inoculated with spores and with mycelium from the fruiting sectors and with mycelium alone from the non-fruiting sectors. These plates were labeled II + and II − respectively. The use of plus and minus to designate different strains of a fungus species is not new. Blakeslee[5] in 1904 applied these terms to sexual strains of the Mucorineae which when bred together produced zygospores. Edgerton[6] in 1914 followed Blakeslee's example and used plus and minus to differentiate strains of Glomerella which when grown together produced the ascogenous stage in abundance.

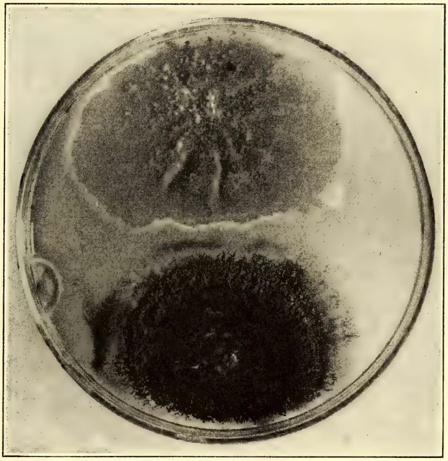

FIG. 2. Plus and minus cultures of Strain II, 7 days old. This plate was inoculated directly from the fruiting and non-fruiting sectors of the plate culture in which the first variation was noticed.

The present paper deals with a fungus reproducing asexually. Plus and minus must therefore not be interpreted as representing male and female strains. Plus is used in the present instance to denote a strain fruiting abundantly and minus to denote a relatively poor fruiting strain.

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[5] Blakeslee, A. F. Proc. Amer. Acad. **40**: 203–321. 1904.

[6] Edgerton, C. W. Plus and minus strains in the genus Glomerella. Amer. Journ. Bot. **1**: 244. 1914.

In the II + colonies on the plates referred to above, the growth was typical of Strain II as it appeared when first isolated. The II − colonies grew rapidly but remained white for 12 days. They then began to take on the olive-green color characteristic of the species and in 20 days from the time of inoculation produced a few large pycnidia about the center of the culture (Figs. 2 and 3). Microscopic examination showed the mycelium, pycnidia and conidia all typical of *C. pirinum*. The − colonies were exactly like Strain I in morphological and cultural features.

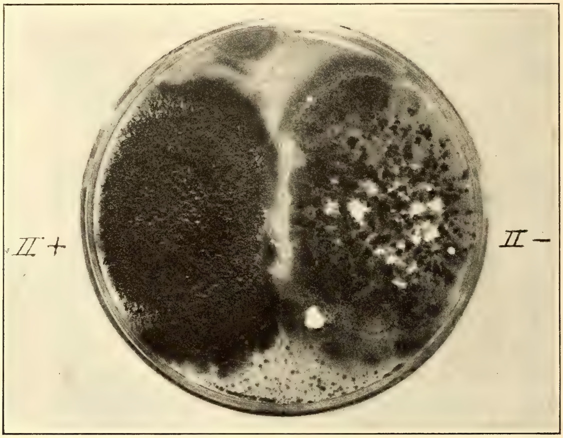

FIG. 3. Same as Fig. 2, 17 days old. Note that II − has produced numerous pycnidia from which the black spore masses are oozing. It was necessary to open the plates when photographing. The contaminations around the edge of the plate got in when the plate was photographed the first time.

It appeared that from Strain II there had arisen a poor fruiting strain identical with Strain I. Poor fruiting is here used in a relative sense. The minus strain produces great multitudes of spores from the few large pycnidia; but, when compared with the enormous numbers of pycnidia and spores produced by the plus strain, the prolificacy of the minus strain falls into insignificance.

To test out the evidence that a minus strain had split off from a plus parent, dilution plates were poured, using spores from both the plus and minus strains. All the progeny of the plus strain were typically plus. All the progeny of the minus strain were typically minus (Figs. 4 and 5). The former produced abundant pycnidia and fruited in 4 days. The latter fruited sparingly only after 14 days. Spores from these dilution cultures were used to pour other plates. Again all progeny of plus parents were typically plus and all progeny of minus parents typically minus. For 12 generations the plus strain remained entirely plus. Then suddenly it gave rise to a minus sector in a petri dish culture. The progeny of this sector were typically minus, whether poured or transferred by wire. The progeny of

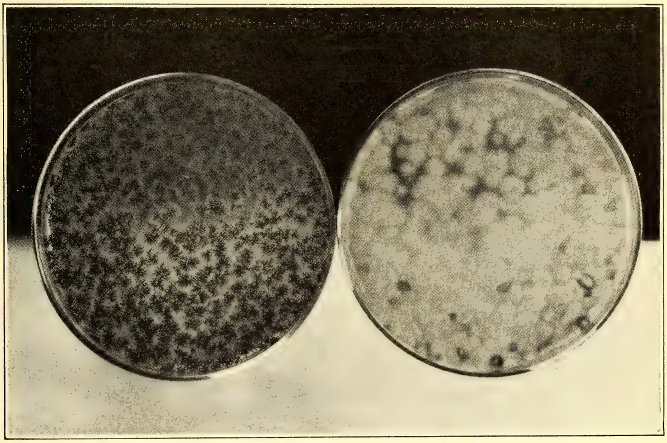

FIG. 4. Second dilution plates poured from the colonies shown in Fig. 3. These plates are 5 days old and of the second generation after the mutation. They therefore have the labels II + 2 and II − 2.

Note: Wherever two or more cultures appear in the same figure they were inoculated at the same time, incubated the same time under the same belljar, and photographed on the same plate.

the plus sectors of the same colony were typically plus and remained so for 5 generations when a third minus strain developed.

Fig. 6 will show better than words the history of the development of these strains.

On November 5, 1914, a culture of *C. pirinum* was received from Mr. J. W. Roberts, of the Bureau of Plant Industry at Washington. It was labeled III and immediately subjected to test to see if it would also develop plus and minus strains.

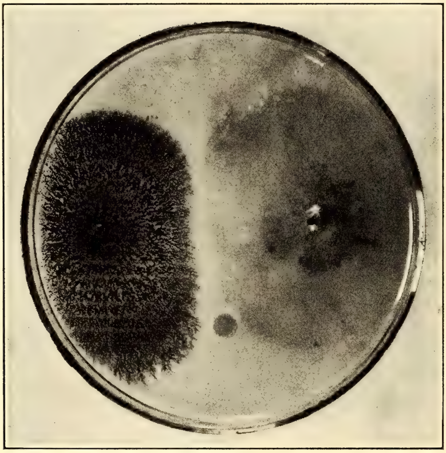

FIG. 5. II + 2 and II − 2 of the same parentage as those in Fig. 4. These cultures are 8 days old. A single large pycnidium has developed at the point of inoculation in the II − 2 colony. When spores alone are transferred the formation of pycnidia begins much later. When mycelium is transferred as in the present case a few pycnidia often develop at an early age due to the fact that young pycnidia are sometimes transferred on the mycelium. These naturally hasten to maturity as soon as food is supplied.

The first set of subcultures was made on Petri dishes of starch agar. Every colony showed fruiting and non-fruiting sectors (Fig. 7). Progeny of the minus sectors have remained constant, for 9 generations or until the present time. Progeny of the plus strain remained constant only 4 generations, when a small minus sector appeared in one of the plus colonies (Fig. 11). Progeny of this minus sector have bred true until the present time.

These results indicate that the *C. pirinum* from the Bureau of Plant Industry was dimorphic in stock culture when received. The separation of the plus and minus forms was accomplished as in Strain

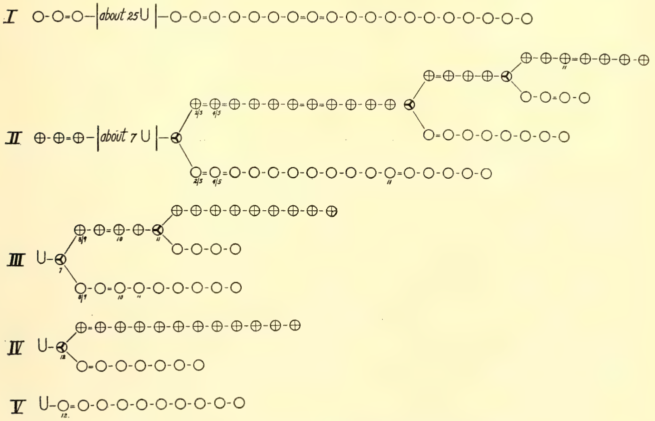

FIG. 6. Chart showing the history of 5 cultures of C. pirinum.

O Petri dish culture of minus strain.

⊕ " " " " plus "

⊗ " " " " mixed strains. It is in such plates that the plus and minus sectors have appeared.

U Test tube culture.

= Succeeding plates poured from spores of preceding culture.

The plus strains suddenly give rise to minus strains which are subsequently constant generation after generation. It will be noticed that fewer generations of minus strains than plus strains have been grown in nearly every case. This is due to the fact that the plus strains fruit more quickly than the minus strains and more generations can be produced in a given time. I and II were isolated by the writer, III (IV) and V by workers of the Bureau of Plant Industry. Subnumbered plates have been photographed. The numbers correspond with the numbers of figures appended.

II. The minus form remained minus. The plus form threw off another minus form in the following 4th generation.

On January 2, 1915, two other cultures of *C. pirinum*, B. P. I. 227 and B. P. I. 345, were received from Dr. J. S. Cooley and designated IV and V respectively. The latter, B. P. I. 345, was isolated by Dr. Cooley from a soft rot of apple on December 3, 1914. The behavior of these cultures is shown in the diagram. Very likely IV is of the same parentage as III, received from Mr. Roberts of the same laboratory. The first subcultures showed it to be dimorphic, *i. e.*, producing plus and minus sectors (Fig. 12).

The first subcultures of V (B. P. I. 345) showed it to be a minus strain (Fig. 12), and it has so remained for 10 generations as shown in the diagram. It is identical with Strain I and with all the other minus strains developed in culture.

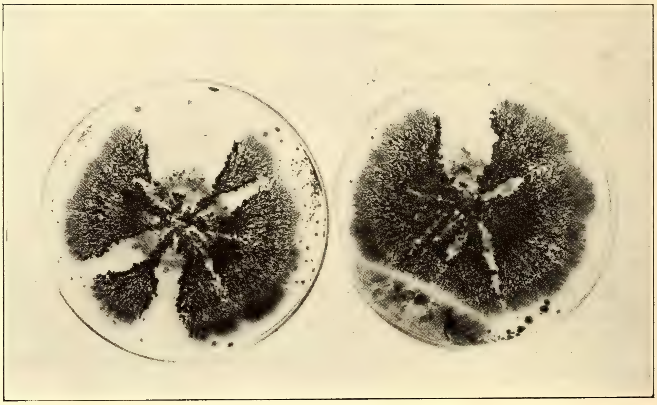

FIG. 7. C. pirinum III. First subculture. 19 days old. Plus and minus sectors were more numerous in these cultures than in any previously or subsequently examined. These plates were opened for examination five days previous to photographing, hence the contaminations around the edges of the cultures.

The plus strains are all identical in appearance and behavior. The fact that the minus strain of *C. pirinum*, viz., I and V, and the plus strain, viz., II and III (IV), have been twice isolated by investigators working separately indicates that plus and minus strains exist in nature.

The cultural studies show that minus strains may arise from plus strains by a sudden sporting or mutation. An objection might be raised that these cultures were impure, *i. e.*, mixtures of two strains. In anticipation of such an idea it seems desirable to state that frequent pourings of dilution cultures were used to preclude such a possibility. Progeny were then selected only from well-isolated plants, microscopic examination of which showed that each was derived from a single spore. By consulting the chart, the frequency of these pourings may be considered. In each instance the poured plate is preceded by the symbol =. It will be evident from this that the possibility of either of the strains being constantly a mixture is eliminated. Both strains have repeatedly arisen from the progeny of a single plus spore. When once purified the minus strains remain constant from generation to generation. The variation apparently is occurring in only one direction.

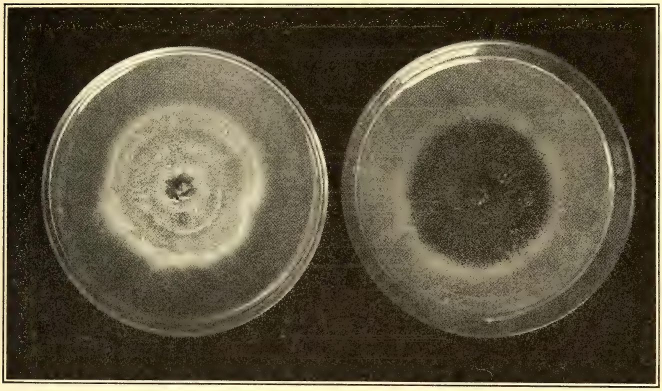

FIG. 8. III + and III −, 8 days old. These plates were inoculated from the plus and minus sectors respectively of the colonies shown in Fig. 7.

Attempts to determine the factor which disturbs the stability of the plus strains and causes the liberation of new minus strains have proved fruitless up to the present time.

Attempts have been made to develop a minus strain from a plus strain by artificial selection alone. The following methods have been employed:

1. Mycelium was continuously selected from the extreme edge of plus colonies where no pycnidia were yet forming.

2. Mycelium was continuously selected from colonies which were subjected to temperatures so low and so high that fruiting was poor.

3. Mycelium was continuously selected from colonies grown in such dry atmosphere that fruiting was poor.

Several generations of such selections gave no promise of the development of a minus strain.

Attempts have also been made to develop a plus strain from a minus strain.

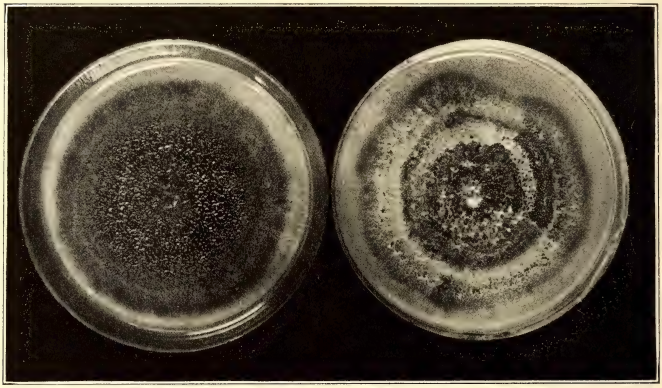

FIG. 9. Same as Fig. 8, 16 days old.

In all the minus strains studied there appear colonies which with age show greater prolificacy in some sectors than in others (Fig. 9). By the transplantation of spores only from the pycnidia of the prolific sectors generation after generation it was thought possible to build up a plus strain. Selection of this sort however has in no way altered the minus strain. Pedigreed cultures after such selection are in no respect different from pedigreed cultures obtained by continuous selection of mycelium from the poorest fruiting sectors of the minus colonies.

These experiments show that selection is not a factor in the origin of the two strains. Since *C. pirinum* reproduces asexually segregation from heterozygous parents cannot explain the origin of the two strains. The only explanation which remains is that the minus strain is a sport or mutant arising from the plus strain at irregular and unprognosticable intervals. What makes it arise and what are the controlling factors of such mutation are worthy of speculation and experiment.

The writer[7] has reported a somewhat similar mutation in a fungus belonging apparently t the genuso Phyllosticta.

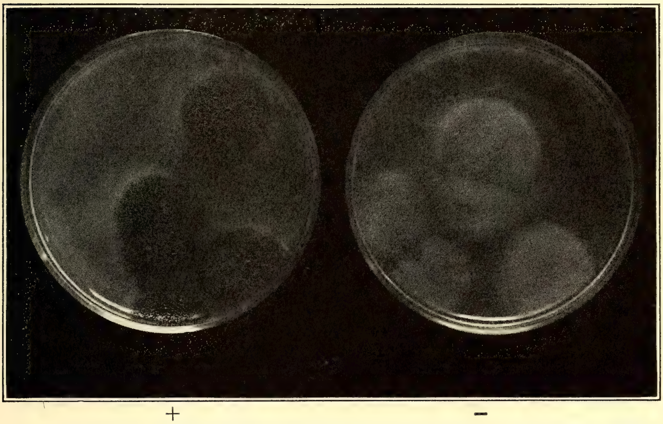

FIG. 10. Third dilution plates of III + 3 and III − 3, 6 days old.

DEVELOPMENT OF PLUS AND MINUS SECTORS

A single isolated plant of *Coniothyrium pirinum* like most fungi grows radially. A germinating spore may produce one or two hyphae which, by a continuous dendritic branching, may give rise to large numbers of branches all of which however are nearly equal in diameter for their entire lengths and many of which end at the growing margin. Each hypha with its many branches then covers an area shaped like the sector of a circle. Therefore if something should happen to a hypha which would change its growth characters, its branches would no doubt also exhibit this change and a sector unlike the rest of the plant would be the result.

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[7] Crabill, C. H. A mutation in Phyllosticta. Phytopathology 4: 396. 1914.

Similarly with a colony. Different spores might give rise to hyphae unlike in their growth characters and sectoring of the colony would result. In the present studies sectoring in a single plant has not been observed. The sectoring has so far always occurred in colonies. Although the data are insufficient for positive proof the

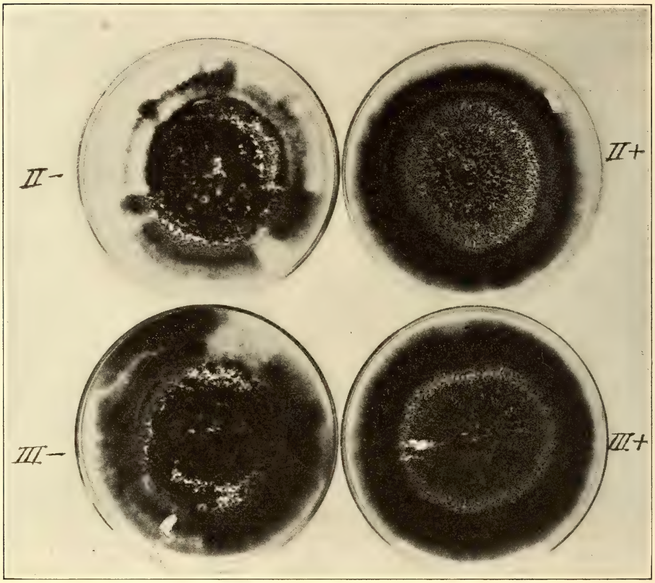

FIG. 11. 24 days old cultures of minus and plus strains of II and III for comparison. II − is in the 11th generation. II + is in the 3d generation after the splitting off of the third minus strain. III − is in the 4th generation. III + is in the 5th generation and shows the small minus sector which gave rise to the 2d minus strain from III. The cultural characters of the two minus and the two plus cultures are identical. In the minus cultures it will be noticed that some sectors produce more pycnidia and more color than others. This fact was taken advantage of in trying to develop a plus strain from a minus strain by selection.

evidence points to the conclusion that the variation which gives rise to minus strains occurs in the spore rather than in the mycelium which develops from it.

The study of plants of *C. pirinum* on Petri dishes shows beyond a doubt that the fungus is chemotactic, *i. e.*, growing only in the direction of its food supply or that it produces some toxic substance detrimental to its own growth.

Whichever the case may be, two plants in close proximity never grow entirely together (Fig. 13). The mycelial threads of the two do not interlace. They are to that extent antagonistic.

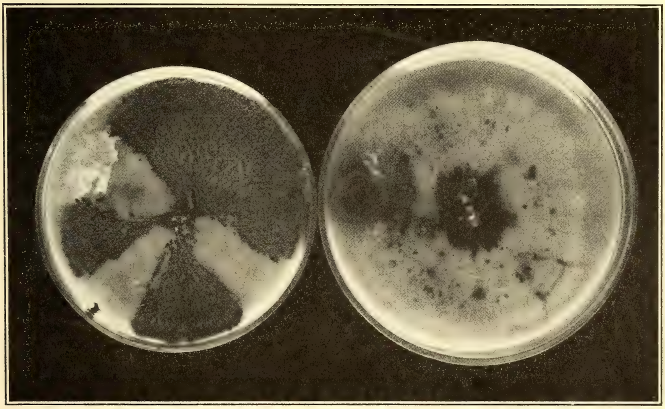

FIG. 12. IV and V, respectively. First sub-cultures, 24 days old.

Some simple tests indicate that this apparent antagonism is not due to excreted toxins. The fungus was grown for two months on a liquid medium containing starch. This medium was then filtered through a sterilized Chamberland filter.

The extract, which was found sterile on plating, was used as follows:

1. A large drop of the plain extract was placed at the margin of a thrifty colony on each of several Petri dishes.

2. Boiled extract was used in a like manner.

3. Plates were poured with plain extract and cooled agar and subsequently inoculated.

4. Boiled extract was used likewise.

In no case was the growth of the fungus hindered by the presence of the extract. It appears then that it is a lack of food rather than a toxic secretion which keeps colonies of *C. pirinum* from growing together.

A microscopic examination of thin Petri dish cultures shows that the mycelial strands exhibit a similar chemotaxis. They tend to diverge continually. If somewhat crowded they grow parallel but seldom converge or cross. It is doubtful therefore if plus mycelium ever crosses over into minus sectors and vice versa. In this way the integrity of the plus and minus sectors is preserved.

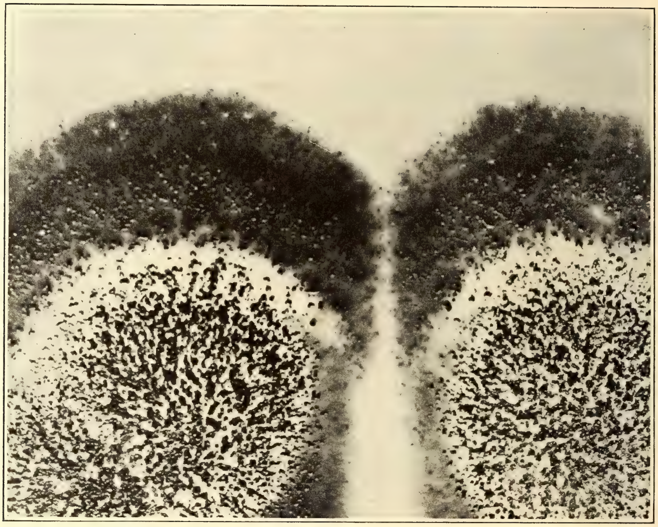

FIG. 13. Two colonies of a plus strain somewhat enlarged. The dark zone is olive green in color and just inside the white advancing margin. Inside of this zone the mycelium is hyaline. The colored oil has been withdrawn from it and deposited in the spores. The two colonies are antagonistic and do not grow together on adjacent sides.

Some experiments were conducted to throw light on this question.

1. Small pieces of agar containing plus and minus mycelium respectively were placed side by side on starch agar. In the resulting growth the two sectors were entirely distinct and typical. The minus portion was in all cases much larger than the plus portion.

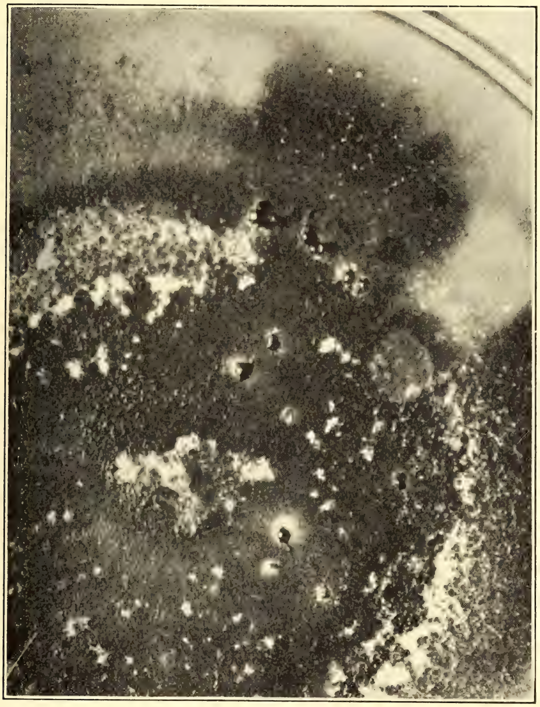

FIG. 14. Culture of a minus strain with the same enlargement as Fig. 13. The mycelium is dark throughout the greater part of the culture. The pycnidia are few, large, producing shining black masses of spores. The greenish color has been removed from the mycelium immediately surrounding the pycnidia. Some white fluffy aerial mycelium is present on the surface.

2. Spores of plus and minus strains were mixed and then used to

inoculate plates. In the resulting colonies sectoring was prominent. The plus sectors were about equal to the minus in number, somewhat less in width and were decidedly distinct as usual.

In some of the photographs appended it is evident that the minus sectors of certain colonies are not quite sector-shaped in outline but narrow somewhat toward the margin (Figs. 7 and 11). As stated above, when pieces of agar containing mycelium of the two were used to inoculate simultaneously the minus sectors were larger than the plus sectors. These observations together with measurements of the rate of growth in colonies of like age indicate that unless the plus strain has a relatively large amount of inoculating material to start from, the minus strain will predominate in growth volume. This is

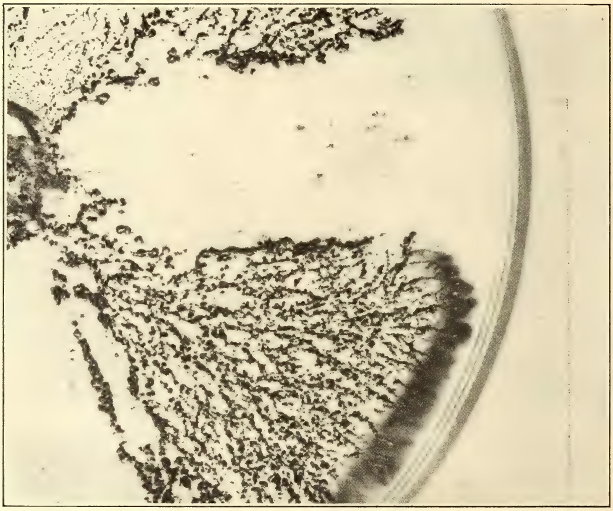

FIG. 15. Enlarged photograph of the IV colony shown in Fig. 12. The mycelium in the minus sector is almost entirely hyaline and fruitless. In the plus sector the color zone which was much broader a few days previous is very narrow and surrounds the outermost ends of the mycelium. Some of the color has diffused out into the agar.

accounted for by the fact that the prolific spore production in the plus strain requires food material and energy which would otherwise be used for vegetative growth.

Color Phenomena and Spore Production in Relation to Temperature

The production of an olive green color is characteristic of the mycelium of *C. pirinum*. On some media color production is more pronounced than on others. The present observations have been made on the two strains growing on starch agar in Petri dishes.

An interesting correlation between color production, sporulation and incubation temperature has been studied. It was found by experience that vegetative growth was most rapid at 25° C., and that sporulation was most active at 18–20° C. It was therefore customary to grow the cultures in moist chambers for 3–6 days at 25° C. and then at 18°–20° C. The moist chambers, each consisting of a plate of water and a tall belljar, maintained an atmosphere of absolute humidity and prevented the drying of the culture medium. The dishes were incubated upside down to prevent moisture from running over the surfaces of the cultures. Under the above conditions the following observations have been recorded.

Plus Strain.—Plus colonies grow rapidly but remain white 2–5 days. In three days pycnidia are usually present in abundance. Most of them are pink at this time but a few may be black due to the dark color of mature spores within. About the 5th day the mycelium just back of the growing ends begins to take on an olivaceous color. Later as the pycnidia mature in abundance this color is all absorbed, leaving the mycelium hyaline (Fig. 13). At the same time more color is formed farther out toward the margin of the radiating colony. The result is that an olivaceous zone migrating continuously outward always exists just behind the growing ends of the mycelium.

Microscopic study shows that the olivaceous mycelium is replete with refringent droplets of oil. In the inner zone of spore production the mycelium is hyaline and contains no oil drops. It is evident that the oil supplies the color. It is manufactured and stored temporarily in the mycelium. Later it is withdrawn and passes into the spores as they develop the dark color characteristic of the species. In cultures only 1–3 days old, spore production precedes color formation

No oil is at that time stored in the mycelium but passed directly into the spores.

In some old cultures the greenish color diffuses out into the agar (Fig. 15). This is especially noticeable in colonies which have reached the edge of the Petri dish in which they are growing. In many cases it has been subsequently absorbed on sporulation.

Cultures 20 days old kept at 13°–15° C. for 10 more days become quite black in the outer zones. This temperature is too low for active production of spores but does not materially hinder the growth and manufacture of oil by the mycelium. The oil is therefore not used as rapidly as produced and necessarily accumulates in the mycelium and imparts to it a dark color.

Cultures incubated constantly at 25° C. produce much less color, as well as fewer pycnidia, than those incubated at lower temperatures.

Minus Strain.—Minus colonies remain white 10 to 20 days. The olive green color then appears in minute spots usually around very young immature pycnidia. The color spreads gradually sometimes appearing more pronounced in some sectors than in others. The pycnidia on maturing absorb the color from the mycelium immediately surrounding them but owing to the fact that these are so few in number, the cultures retain most of the olive color which darkens with age until the culture is quite black (Fig. 14). Minus cultures incubated constantly at 25° C. produce very little color. The optimum temperature for spore production is 18°–20° C. Some cultures will scarcely fruit at all at 25° C. but fruit typically at 18°–20° C. Color production and sporulation are both maximum at the latter temperatures.

Mixed Cultures.—In mixed cultures in which plus and minus strains grow in respective sectors, the plus sectors develop and later absorb color as described for pure plus strains. The minus sectors on the other hand may never develop this color or if they do it is in small amounts and very tardy in appearing (Fig. 15). In many cases there is no spore production whatever. Normally a few pycnidia appear with characteristic tardiness.

INOCULATIONS

The plus and minus strains of *Coniothyrium pirinum* have been used to inoculate apple leaves. Holes were seared in the leaves with a hot needle, wetted and smeared with spores. The leaves were then bagged.

In two weeks the spots inoculated with each strain turned whitish and bore several pycnidia from which it was easy to reisolate the strain. The cultures show that both the strains retained their diagnostic characteristics. One month after inoculation no enlargement of the seared spots has taken place. In fact some of the dead tissue bearing pycnidia of the fungus has fallen out producing a "shot hole" effect. This is further evidence that *Coniothyrium pirinum* is a saprophyte. The above experiment was conducted under controlled conditions in the greenhouse.

CONCLUSIONS

1. *Coniothyrium pirinum* is sometimes dimorphic in culture and probably also in nature.

2. Two distinct strains have been isolated, viz., a plus strain, which fruits abundantly, and a minus strain, which fruits poorly.

3. The minus strain arises in artificial culture by sudden sporting from the plus strain. This phenomenon has been observed in four separate instances.

4. Minus strains never give rise to plus strains but remain constant, generation after generation.

5. Attempts to develop the strains from each other by continuous selection of extremes have been unsuccessful.

6. Attempts to determine the cause of the sporting have been fruitless.

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BLACKSBURG, VA.

THE GENUS ESPELETIA *

PAUL C. STANDLEY

The genus Espeletia is a member of the Asteraceae, placed by Hoffman in the tribe Heliantheae, subtribe Melampodinae, in his treatment of the family in Engler and Prantl's Natürlichen Pflanzenfamilien.[1] Although it has often been referred to other groups, systematists are now agreed as to its proper disposal. The plants are among the most conspicuous of the composites, because of the long wool which closely invests the leaves and inflorescence of most species, and because of the peculiar habit of growth of some members of the genus. The woolly covering is not peculiar to the Espeletias, however, some species of the genus Culcitium, for example, a member of the Senecioneae, closely simulating in their general appearance certain species of the present genus.

Two species of Espeletia are tall, much branched shrubs. Others have a large tuft of radical leaves borne upon the surface of the ground from which one or more flowering stems rise. A few, like *Espeletia grandiflora*,[2] develop tall, thick, erect caudices a meter high or more, which are naked below but bear at the summit a large cluster of leaves from which several flowering stems rise. In their habit of growth this group suggests some of the Senecios and Lobelias which abound in similar situations in the high mountains of East Africa.[3] The species are not numerous and are confined, so far as known, to the páramos of the high cordilleras of Colombia and Venezuela, occurring usually at elevations of 3,000 meters or more. The southernmost species, *E. corymbosa*, was collected not far from the southern border of Colombia, and it is not improbable that it or some related species may occur in Ecuador.

The genus Espeletia was founded by Humboldt and Bonpland in 1809,[4] three species being described and illustrated. The name was given in honor of Don José de Espeletia, at one time viceroy of the Kingdom of New Grenada, who encouraged botanical explorations about Bogotá during his term of office. Bonpland expressly states that the generic name should be credited to Mutis, who discovered *E. grandiflora* in the vicinity of Bogotá. It is thus apparent that *Espeletia grandiflora* is the type of the genus,—a matter of no very great importance, since only one species has ever been referred to any other genus. Libanothamnus was based by Ernst upon the plant now known as *Espeletia neriifolia*, which differs in habit from other members of the group and had previously been referred to two other genera. Ernst did not recognize the relationship of Humboldt's *Trixis neriifolia* with Espeletia, nor was he aware that Weddell had already transferred it to its proper position, otherwise he probably would not have made it the type of a new genus.

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* Published by permission of the Secretary of the Smithsonian Institution.

[1] 4^5: 216. 1890.

[2] See Engl. & Prantl, Pflanzenfam. 4^5: *f. 109*.

[3] See the National Geographic Magazine **27**: 194, 196, 197, 200. 1915.

[4] Pl. Aequin. **2**: 9.

No additions were made to Espeletia after 1809 until Weddell published his elaborate treatment of the Andine flora in 1855.[5] That author lists eleven species, seven of which are described as new, while one is transferred from another genus. Since 1855 the only published addition to Espeletia is a subspecies described in 1905.

That so little concerning the genus is found in later literature results from the fact that few botanists have visited the Andine regions of Venezuela and Colombia in recent years. Lately the U. S. National Herbarium has received twenty-two sheets of Espeletia, representing nearly as many collections. This is probably more material than is found in any other herbarium and has led the writer to undertake a review of the genus as a whole, in the course of which no less than six species apparently new have been discovered. Nearly all these sheets were included in a large set of Venezuelan plants collected by Dr. Alfredo Jahn, mainly in the high cordilleras, in regions difficult of access and not visited by any other recent collector. The collection includes many highly interesting plants, but no genus is so completely represented as the present one. Of the species of Espeletia previously described from this region Doctor Jahn has recollected all but two, and one of these is a plant of doubtful standing.

Of the shrubby species of Espeletia one is known as "incienso," since the branches produce a large quantity of resin which is burned as incense in the churches. The non-shrubby species are commonly known as "frailejón," their dense covering of white or gray wool recalling to the Venezuelans the cloaks of the brothers of some of the religious orders. Humboldt and Bonpland state that the resin of *E. grandiflora* was used at Bogotá in the manufacture of printers' ink, a purpose for which it was highly prized. The resin, they state, was known at Bogotá as "trementina (turpentine)," although it did not possess either the odor or the consistency of the turpentine of commerce.

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[5] Chloris Andina, Essai d'une flore de la région alpine des cordillères de L'Amérique du Sud, in Castelnau, Expédition dans les parties centrales de l'Amérique du Sud, part 6.

KEY TO THE SPECIES

- Shrubs or trees with much branched, woody stems.
  - Leaf blades sinuately and subspinosely denticulate. 1. *E. banksiaefolia.*
  - Leaf blades entire. 2. *E. neriifolia.*
- Herbs, often with much elongated, erect caudices, but these simple, never with much branched, woody stems.
  - Heads usually solitary on each scape, rarely more than one.
    - Leaf blades linear or narrowly ligulate, attenuate, 25 to 30 cm. long; heads 4 to 4.5 cm. in diameter; pubescence rufous or fulvous. 3. *E. moritziana.*
    - Leaf blades linear-oblong, 4 to 6 cm. long; heads about 2 cm. in diameter; pubescence nearly white. 4. *E. weddellii.*
  - Heads several or numerous, the stems not scapose.
    - Cauline leaves opposite; bracts of the inflorescence often opposite or verticillate.
      - Pubescence of stems and leaves white, silvery; heads radiate or discoid; basal leaves 4 cm. wide or less.
        - Bracts of the inflorescence verticillate; leaves 3 to 4 cm. wide. 7. *E. argentea.*
        - Bracts alternate; leaves 1.8 cm. wide or less. 8. *E. grisea.*
      - Pubescence usually fulvous or rufescent; heads radiate; basal leaves 4 to 10 cm. wide.
        - Involucral bracts broadly ovate; nerves of the leaves mostly obscured by the wool. 5. *E. grandiflora.*
        - Involucral bracts lanceolate or narrowly ovate; nerves of the leaves prominent. 6. *E. schultzii.*
    - Cauline leaves and bracts of the inflorescence all alternate, rarely subopposite.
      - Heads in an elongated simple raceme. 9. *E. spicata.*
      - Heads in a more or less compound panicle or corymb.
        - Leaf blades of nearly uniform width throughout, not noticeably narrowed toward the base.
          - Leaf margins strongly revolute, the blades 3 to 6 mm. wide, soon glabrate on the upper surface; inflorescence congested. 10. *E. jahnii.*

Leaf margins flat or slightly revolute, the blades 7 to 12 mm. wide; inflorescence openly corymbose or paniculate.

Heads 12 mm. broad, their pubescence brown, short. 11. *E. pannosa.*

Heads 20 to 25 mm. broad, their pubescence white, very long. 12. *E. floccosa.*

Leaf blades evidently broadest above the middle, conspicuously narrowed at the base.

Leaves sericeous beneath, with short appressed hairs; heads about 8 mm. broad. 13. *E. paltonioides.*

Leaves more or less lanuginous beneath, with loose hairs; heads more than 8 mm. broad.

Leaves in age glabrous on the upper surface or sparsely pubescent.

Heads 25 mm. broad; leaf blades narrowly oblong, attenuate at the apex, slightly narrowed below; involucral bracts glanduliferous but nearly without pubescence. 14. *E. lindenii.*

Heads about 12 mm. broad; leaf blades elliptic, attenuate below to a winged petiole; involucral bracts copiously pilose. 15. *E. bracteosa.*

Leaves copiously lanuginous on the upper surface.

Involucral bracts ovate or elliptic-ovate, sparsely pilose outside. 16. *E. corymbosa.*

Involucral bracts lanceolate, densely pilose outside. 17. *E. funckii.*

1. Espeletia banksiaefolia Schultz Bip. & Ettingh.; Wedd. in Cast. Expéd. Amér. Sud Bot. 1: 67. 1855

Type locality: Sierra Nevada de Mérida, Venezuela, at an altitude of 3,500 meters. Type collected by Funck and Schlim (no. 1550).

This differs from all other species in having denticulate leaves. Weddell stated that he had seen only a fragment of the plant. It is more than possible that it has been referred to the wrong genus.

2. ESPELETIA NERIIFOLIA (H. B. K.) Schultz Bip.; Wedd. in Cast. Expéd. Amér. Sud Bot. 1: 67. 1855

Trixis neriifolia Humb. Voy. Rég. Équin. Rel. 1: 605. 1814.
Bailleria ? neriifolia H. B. K. Nov. Gen. & Sp. 4: 289. 1820.
Clibadium ? neriifolium DC. Prodr. 5: 507. 1836.
Libanothamnus neriifolia Ernst, Vargasia 186. 1870.

TYPE LOCALITY: Silla de Caracas, Venezuela. Type collected by Bonpland.

Weddell reports several collections from the Silla de Caracas and the Sierra Nevada de Mérida, Venezuela. A specimen in the U. S. National Herbarium was collected by Otto Kuntze in 1877 at the type locality. In May, 1913, Mr. Pittier collected the plant in the upper belt of the Pico de Naiguatá, State of Miranda, at an altitude of 2,400 to 2,765 meters. The common name is "incienso."

The species differs from most others in being a shrub 3 to 4 meters high with numerous leafy branches. The heads are very numerous, in broad corymbs, and comparatively small, 1 cm. broad or less. They are nearly destitute of the long wool characteristic of most of the other species. Upon this plant Ernst founded his genus Libanothamnus ("incense tree"). He erred in referring it to the Senecioneae and was apparently unaware that Weddell had previously referred it to Espeletia. The shrub differs so conspicuously in habit from other members of the genus, with some of which Ernst must have been acquainted, that it is not surprising that he did not associate it with Espeletia.

3. ESPELETIA MORITZIANA Schultz Bip.; Wedd. in Cast. Expéd. Amér. Sud Bot. 1: 65. 1855

TYPE LOCALITY: Sierra Nevada de Mérida, Venezuela, at an altitude of 4,200 to 4,500 meters. Type collected by Moritz (no. 1416).

Two collections of this have been secured by Doctor Jahn, one in the Páramo de Timotes, State of Táchira, at an altitude of 3,000 to 3,500 meters (no. 150); and the other in the Páramo de la Culata, Cordillera de Mérida, at an altitude of 3,500 meters (no. 235). The common name is given as "frailejón dorado."

This species, like *E. weddellii*, is characterized by monocephalous stems. The heads are much larger than in that species, however, being 4 to 5.5 cm. in diameter. The bracts are covered with very long and dense pubescence, the unopened heads appearing to be globular masses of wool.

Weddell says the heads are "perpaucis, interdum solitariis," and in the present specimens they are always solitary. The following notes may be added from the recent collections: Pubescence ferruginous, especially on the younger leaves; leaves 25 to 30 cm. long, 8 to 14 mm. wide, coriaceous; flowering stems stout, 40 to 60 cm. high; paleæ of the disk linear or oblong-linear, acuminate, minutely serrulate near the apex; achenes 2 to 2.5 mm. long, nearly black, smooth, sharply angled.

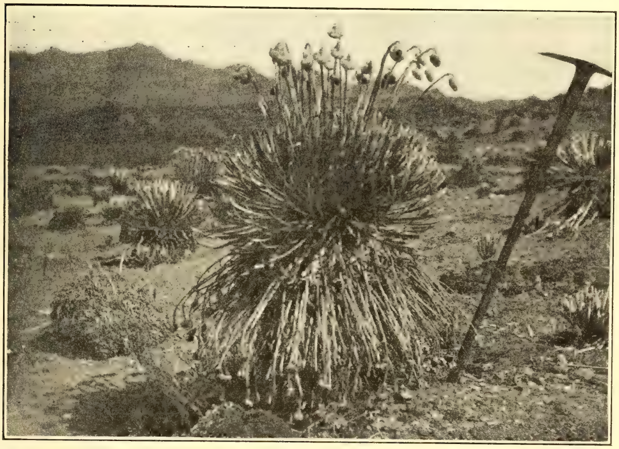

FIG. 1. *Espeletia moritziana.* Páramo de Timotes, Venezuela. Photo by Dr. A. Jahn.

The inner involucral bracts are longer than in any other species, their apices being long-attenuate.

4. ESPELETIA WEDDELLII Schultz Bip.; Wedd. in Cast. Expéd. Amér. Sud Bot. 1: 66. *pl. 15, B.* 1855

TYPE LOCALITY: "Venezuela: Páramo de Niquitao!, dans les Andes de Truxillo, á une élévation de 4,000–4,500 mètres." Type collected by Linden (no. 1443).

Two specimens of this are in Doctor Jahn's collections, one from the Páramo de Timotes, State of Táchira, at an altitude of 3,000 to 3,500 meters (no. 152); and the other from the type locality, the Teta de Niquitao, the highest peak of the Sierra Nevada de Mérida, at an altitude of 3,000 to 4,000 meters (no. 151). The common name is given as "frailejón de batata." The word batata (tuber or bulb) refers to the large tuber-like roots which, however, are not present upon the specimens.

The species is distinguished by its very numerous short leaves which form a dense basal cluster, and by its monocephalous stems. Weddell says that it is the lowest plant of the genus and this is probably true. From the specimens it can be seen that the caudex is not elongated and that the leaves rest upon the ground.

Weddell states that there are one or two heads on each stem. In these recent specimens there is never more than one. The leaves are described as "acutatis" but the tips are very blunt, and Weddell so figures them. In Doctor Jahn's specimens the leaves are 4 to 6 cm. long and 5 to 10 mm. wide; the flowering stems are 6 to 30 cm. long, evidently elongating in age; the paleæ of the disk are oblong-linear, densely villous at the apex, and bearded at the base with long, white, erect hairs; the achenes, which seem not to be full developed, are about 2.5 mm. long.

5. ESPELETIA GRANDIFLORA Humb. & Bonpl. Pl. Aequin. 2: 9. *pl. 70.* 1809

Espeletia hartwegiana Schultz Bip.; Wedd. in Cast. Expéd. Amér. Sud Bot. 1: 62. 1855, as synonym.

Espeletia oppositifolia Schultz Bip.; Wedd. loc. cit., as synonym.

TYPE LOCALITY: Near Bogotá, Colombia. Type collected by Bonpland.

ILLUSTRATIONS: Wedd. in Cast. Expéd. Amér. Sud Bot. 1: *pl. 15, A.*

Collected by Mr. H. Pittier in the Páramo de Buena Vista, Huila Group, Central Cordillera, State of Cauca, Colombia, at an altitude of 3,000 to 3,600 meters, in January, 1906 (no. 1116). Weddell[1] reports several collections from Colombia and a single one from the Sierra Nevada de Mérida, Venezuela (*Linden* 398, in part).

Schultz's manuscript name *hartwegiana* was based, doubtless, upon a specimen collected by Hartweg (no. 1137) in the Páramo de

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[1] In Cast. Expéd. Amér. Sud Bot. 1: 63. 1855.

Guanacas, in the Andes of Popayán, Colombia. The name *oppositifolia* was based upon Linden's no. 398 from the Sierra Nevada de Mérida, Venezuela. Weddell states that this collection is a mixture, the inflorescence (from which the specific name was taken) being of *E. grandiflora* and the leaves those of *E. moritziana*.

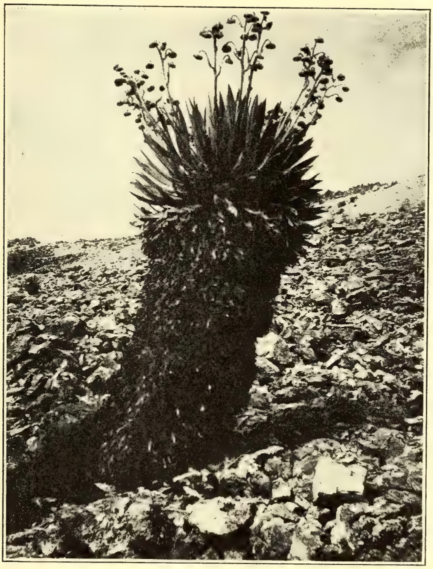


FIG. 2. *Espeletia grandiflora.* Páramo de Culata, Venezuela. Photo by Dr. A. Jahn.

6. ESPELETIA SCHULTZII Wedd. in Cast. Expéd. Amér. Sud Bot. 1: 63. 1855

TYPE LOCALITY: Páramos of the Cordilleras of Mérida, Venezuela, at an elevation of 3,200 to 3,500 meters. Type collected by Linden (no. 370). Weddell also cites Moritz's no. 1419 from the same region.

Three of Doctor Jahn's collections are referred here: no. 245, from La Culata, Cordillera de Mérida, altitude 3,500 meters; no. 236, from Pan de Azúcar, Cordillera de Mérida, altitude 4,000 meters, and no. 153, from the Páramo de Timotes, State of Táchira, altitude 3,000 to 3,500 meters. The common name is given as "frailejón." The leaves are said to be used for wrapping cheese and butter, to secure the flavor and aroma which they impart.

The species appears to be very closely related to *E. grandiflora*, and Weddell's diagnoses do not afford a satisfactory means of separating the two. *E. schultzii* has paler wool, however, more conspicuously veined leaves, narrower involucral bracts, and presumably a broader inflorescence. Whether this last distinction will hold when a large series of specimens is brought together is doubtful. The leaves are almost ligulate, not "oblongo-lanceolatis" as Weddell described them in his diagnosis, a characterization which is somewhat amended in his discussion of the species. In the present specimens they are 18 to 31 cm. long and 2.5 to 6 cm. wide, being scarcely at all narrowed at the base, slightly broadest above the middle, and from obtuse to acute. The heads vary considerably in size with age, ranging from 2 to 3.5 cm. in diameter. The paleæ of the disk are nearly linear, attenuate, furnished near the apex with numerous short, stiff hairs.

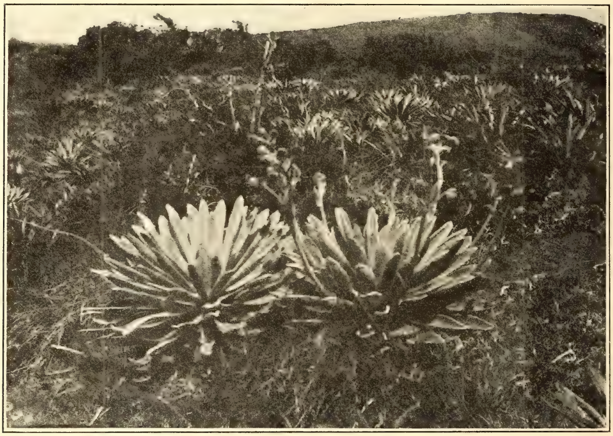


FIG. 3. *Espeletia schultzii.* Páramo de la Cristalina, Venezuela. Photo by Dr. A. Jahn.

7. Espeletia argentea Humb. & Bonpl. Pl. Aequin. 2: 12. *pl. 71.* 1809

Type locality: Near Zipaquira, north of Bogotá, central Colombia, at an altitude of 1,300 meters. Type collected by Humboldt and Bonpland.

Weddell reported two forms of this species: The first "capitulis radiatis," and the second "capitulis discoideis," the typical form, known only from the type collection. To the first form, with radiate heads, he referred three specimens from the mountains of Mérida, Venezuela, at elevations of 3,200 to 3,900 meters, collected by Linden (no. 401), Funck and Schlim (no. 1072), and Moritz (no. 1418). He cited as a synonym *Espeletia nivea* Moritz, mss., this name doubtless referring to Moritz's specimen. It is probable that none of the Venezuelan specimens are really *E. argentea,* but belong rather to *Espeletia floccosa, E. grisea,* or *E. pannosa,* which are described as new in the present paper. To which one of these three the name *nivea* was applied can not be determined without examination of Moritz's specimens.

Bonpland illustrated and described the heads as discoid. From his diagnosis and plate we also find that the leaves are opposite and the bracts of the inflorescence opposite or verticillate. Weddell describes the cauline leaves as "interdum oppositis," this modification of the original description probably necessitated by the inclusion of the Venezuelan specimens. Certain other modifications of Bonpland's diagnosis introduced by Weddell into his notes seem to indicate that he had before him perhaps the plant described here as *Espeletia pannosa.*

8. **Espeletia grisea** sp. nov.

Caudex stout, 20 cm. long, 2 to 2.5 cm. in diameter, from a stout elongated tap-root, covered with closely imbricated leaf bases; radical leaves narrowly linear-oblanceolate, 15 to 21 cm. long, 13 to 18 mm. wide, acuminate at the apex, gradually and slightly narrowed toward the base, dilated at the point of insertion, subcoriaceous, flat, or the margins slightly revolute, abundantly lanate on both surfaces with grayish hairs but more densely so beneath, the midrib not prominent, the lateral veins scarcely perceptible; flowering stem about 120 cm. high, stout, densely lanate below, the pubescence less abundant above and somewhat deciduous, corymbosely much branched, the branches long, ascending or erect, slender, the stems furnished below with several pairs of opposite leaves, these similar to the basal ones but smaller; branches of the inflorescence all alternate, the bracts resembling the cauline leaves but smaller; heads very numerous, on slender peduncles 2 to 4 cm. long, these viscid and rather sparsely tomentose with long loose whitish hairs and shorter brown ones; heads 15 mm. broad in age, subglobose, the outer paleæ of the receptacle and the involucral bracts reflexed; involucral bracts narrowly lanceolate to narrowly obovate, acute, pilose outside with brown hairs, glabrous within; paleæ of the disk cuneate-obovate, acutish, densely viscid-pilose outside near the apex; corolla tube sparsely villous outside; achenes not seen.

Type in the U. S. National Herbarium, no. 602351, collected on the Sierra Nevada de Mérida, State of Mérida, Venezuela, at an altitude of 3,000 to 4,000 meters, in January, 1911, by Dr. Alfredo Jahn (no. 157). Additional material of the same collection, consisting of a caudex and basal tuft of leaves, is mounted on sheet no. 602352.

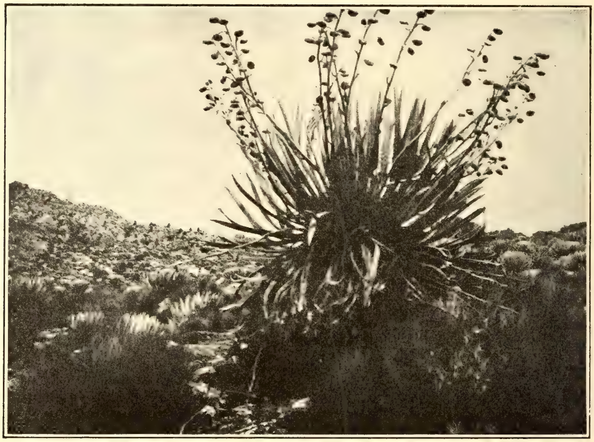


FIG. 4. *Espeletia spicata.* Sierra Nevada de Mérida, Venezuela. Photo by Dr. A. Jahn.

Espeletia grisea is evidently related to *E. argentea* but is distinguished by the shorter, narrower leaves (which are less narrowed toward the base), the villous corolla, and the alternate branches and bracts of the inflorescence. The heads are too far developed to determine whether they are radiate or discoid.

9. Espeletia spicata Schultz Bip.; Wedd. in Cast. Expéd. Amér. Sud Bot. 1: 65. 1855

Type locality: Sierra Nevada de Mérida, Venezuela, at an altitude of 4,500 meters. Type collected by Linden (no. 400).

Collected by Doctor Jahn on the Sierra Nevada de Mérida, at an altitude of 3,000 to 4,000 meters (no. 158); on Pan de Azúcar, Cordillera de Mérida, at an altitude of 4,000 meters (no. 237); and in the Páramo de Timotes, State of Táchira, at an altitude of 3,000 to 3,500 meters (no. 149). In the first specimen the inflorescence is lacking, but the leaves show that it is of this species. The second specimen bears only part of an inflorescence, with a few heads about 15 mm. in diameter; the leaves are 27 to 40 cm. long and 18 to 27 mm. wide; and the whole plant is densely woolly with fulvous hairs. The third specimen shows a complete inflorescence about 95 cm. high; the stem is very stout and densely lanuginous; the peduncles average about 5 cm. in length, and the heads 3 to 3.5 cm. in diameter.

10. **Espeletia jahnii** sp. nov.

Leaves all basal, 18 to 35 cm. long, 3 to 6 mm. wide (excluding the strongly revolute margins), linear or nearly so, acute or acuminate at the apex, widened at the sheathing base, coriaceous, the margins strongly revolute, densely clothed on the upper surface with long, white, nearly straight wool when young but becoming glabrate in age, densely tomentose on the lower surface, the bases of the leaves more densely furnished with wool; flowering stems stout, 20 to 25 cm. high, naked up to the inflorescence, covered by a dense, matted, slightly fulvous wool; bracts of the inflorescence 2 to 5.5 cm. long, similar to the leaves but broader; inflorescence corymbose, of 12 to 15 crowded, short-pedunculate heads, these about 1 cm. in diameter; involucral bracts few, lanceolate, obtuse or acutish, densely covered with long wool; paleæ of the disk 3.5 mm. long, linear, slightly broadened upward, densely glandular near the apex; rays wanting; styles much exserted.

Type in the U. S. National Herbarium, no. 602482, collected in the Páramo del Batallón, State of Táchira, Venezuela, at an altitude of 3,000 meters, in March, 1911, by Dr. Alfredo Jahn (no. 155).

This is well distinguished from all the other species by the very narrow leaves which are soon glabrate on the upper surface and strongly revolute. The flowering stems, too, are lower than in most species. It is not possible to determine from the specimens whether the plant has an elongate, erect caudex.

In the Páramo de las Rosas, State of Trujillo, at an altitude of 3,200 meters, Doctor Jahn collected another plant which somewhat resembles this, the leaves being slightly broader and glabrous on the upper surface. Unfortunately this specimen is without flowers. It is doubtless an undescribed species.

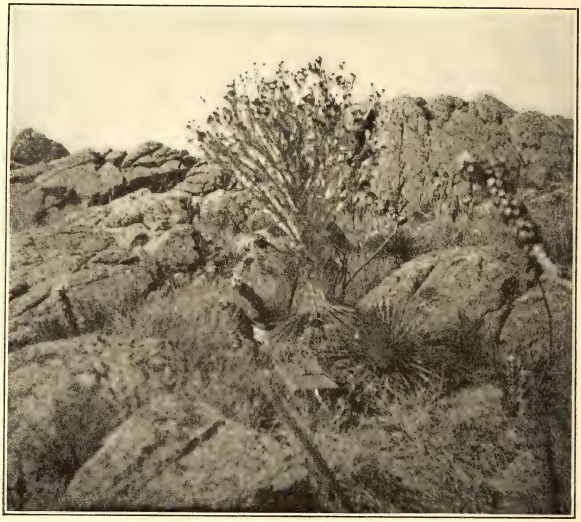


FIG. 5. *Espeletia jahnii.* Páramo Malpaso, State of Táchira, Venezuela. Photo by Dr. A. Jahn.

11. **Espeletia pannosa** sp. nov.

Caudex short, about 9 cm. long, 8 cm. wide at the crown, from a very thick ligneous root, bearing very numerous erect leaves; radical leaves broadly linear, of uniform or nearly uniform width throughout, except for the slightly dilated point of attachment, 16 to 30 cm. long, 7 to 8 mm. wide, coriaceous and rigid, the margins slightly or strongly revolute, obtuse to acute at the apex, densely covered on the upper surface with long, straight but somewhat matted, silvery white hairs, densely tomentose beneath with long, matted, white or slightly yellowish hairs, the midrib very broad and prominent beneath, the lateral nerves not apparent; flowering stem 45 cm. high or more, stout, bearing a few alternate leaves similar to the basal ones but much smaller, corymbosely branched above, the branches few, erect or nearly so, densely tomentose with dark brown hairs, the pubescence of the lower part of the stem lanate, white; heads rather numerous, 12 to 15 mm. broad, on stout erect peduncles 2 to 5 cm. long, these densely tomentose with brown hairs; bracts of the inflorescence 1 to 2 cm. long, oblong-lanceolate, alternate; involucral bracts numerous, linear-lanceolate to narrowly ovate, acute or acuminate, glabrous within, densely villous outside with long matted brown hairs; rays numerous, 5 to 6 mm. long, shallowly bilobate at the apex; paleæ of the disk densely villous outside; corolla lobes densely pilose; achenes 1.5 mm. long, obtusely angled, dark brown.

Type in the U. S. National Herbarium, no. 602484, collected in the Páramo del Jabón, State of Trujillo, Venezuela, at an altitude of 3,000 to 3,200 meters, in October, 1910, by Dr. Alfredo Jahn (no. 165).

This is related to *Espeletia argentea* but is easily distinguished by the radiate heads, narrow radical leaves, and alternate cauline leaves. It is a handsome plant by reason of its silvery leaves which contrast with the brown pubescence of the inflorescence.

Closely related to this is a specimen collected by Doctor Jahn (no. 244) at La Culata, Cordillera de Mérida, at an altitude of 3,500 meters. The form of the leaves is almost exactly the same as in *E. pannosa*, except that they are 40 cm. long and acuminate. The inflorescence is represented only by a few lateral branches, each subtended by a large, silvery bract, and terminated by several sessile heads. This specimen probably represents an undescribed species, but it is not complete enough to determine all the essential characters.

12. **Espeletia floccosa** sp. nov.

Radical leaves broadly linear, of nearly uniform width throughout, slightly dilated at the point of attachment, 30 cm. long, 9 to 11 mm. wide, acute or acuminate, coriaceous, rigid, erect, flat, densely sericeous on the upper surface with long, matted, silvery white hairs, densely tomentose on the lower surface with matted, white or yellowish hairs, the midrib large and prominent beneath, the lateral nerves not visible; flowering stem nearly a meter high, very stout, bearing a few long alternate leaves below, corymbosely branched above, the branches numerous, stout, erect, alternate, densely covered throughout with very long white wool, this loose and floccose; heads numerous, 18 to 25 mm. in diameter, on stout peduncles 1 to 4 cm. long; involucral bracts numerous, linear, attenuate, 10 to 15 mm. long, glabrous within, densely covered outside with long loose white wool; paleæ of the disk narrowly linear, rigid, erect, 5 to 6 mm. long, acute, pubescent outside; corolla villous, white; achenes 2.5 mm. long, compressed, very broad, obtusely angled.

Type in the U. S. National Herbarium, no. 602483, collected in the Páramo del Jabón, State of Trujillo, Venezuela, at an altitude of 3,000 to 3,200 meters, in October, 1910, by Dr. Alfredo Jahn (no. 154). The collector gives the common name as "frailejón plateado," and states that the flowers are white. The heads have been badly eaten by insects, and if any rays were present they have disappeared.

The collector states that the same plant was observed in the Páramo de Timotes and the Sierra Nevada de Mérida. It may be, however, that this was not distinguished from *E. grisea* and *E. pannosa.*

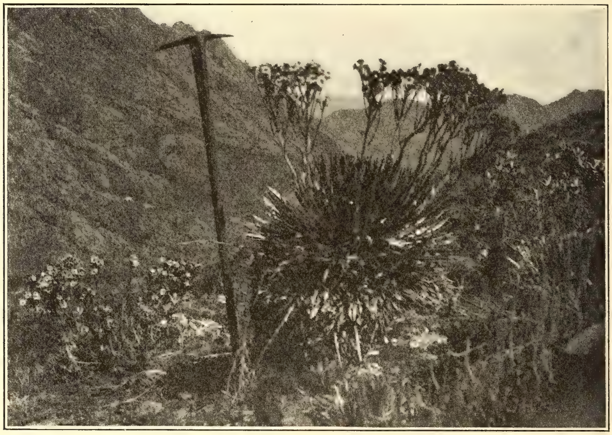


FIG. 6. *Espeletia floccosa.* Sierra Nevada de Mérida, Venezuela. Photo by Dr. A. Jahn.

Espeletia floccosa is a most distinct species, especially because of the abundant, long, white wool. The paleæ of the disk are narrower than in any other species. The plant is related, perhaps, to *E. argentea,* but it has much larger heads, longer, narrower bracts, and narrower leaves.

13. **Espeletia paltonioides** sp. nov.

Caudex elongated, stout, 3.5 cm. in diameter, densely covered with leaves, their bases imbricated; leaf blades narrowly linear-oblanceolate, 20 to 28 cm. long, 13 to 18 mm. wide, attenuate to the apex, long-attenuate to the broadly margined petiole, this abruptly dilated at the point of attachment, the margins strongly revolute, the upper surface grayish green, closely covered with very short, stiff, yellowish, almost scabrous hairs, densely sericeous on the lower surface with very dense, short, white or fulvous, appressed hairs, the blades coriaceous but not much thickened, the midvein very prominent beneath, the lateral veins inconspicuous, irregular, somewhat reticulate; flowering stem tall, erect, densely sericeous, furnished with numerous alternate bracts or leaves; inflorescence of many heads in a rather compact corymb 16 cm. high and 12 cm. broad, the branches 3.5 to 11 cm. long, the heads corymbose at the end of each branch, on peduncles 2 to 12 mm. long; heads 8 to 10 mm. broad; bracts few, oblong-linear to narrowly oblong, acute, glandular-puberulent at the apex, densely pubescent with mostly appressed hairs, these abraded by weathering; paleæ of the disk oblong or oblong-spatulate, rounded or subtruncate at the apex, puberulent, glandular above; corolla 3 to 3.5 mm. long, glabrous; achenes 2.5 mm. long, sharply trigonous.

Type in the U. S. National Herbarium, no. 602354, collected in the Páramo de las Rosas, State of Trujillo, Venezuela, at an altitude of 3,200 meters, in October, 1912, by Dr. Alfredo Jahn (no. 159).

The material consists of a stout caudex bearing a thick cluster of leaves and a weathered inflorescence. Unfortunately, because of the age of the latter, it is impossible to describe the pubescence of the involucral bracts or the form of the rays, if these be present. All the leaves and bracts of the inflorescence have disappeared, too. Nevertheless, this is easily distinguished from all other species by the short, close pubescence of the leaves. The heads are very numerous and small. The species is most closely related, probably, to *E. corymbosa.* The form of the leaves suggests the fronds of a fern, *Paltonium lanceolatum* (L.) Presl; hence the specific name.

14. ESPELETIA LINDENII Schultz Bip.; Wedd. in Cast. Expéd. Amér. Sud Bot. 1: 66. 1855

TYPE LOCALITY: Páramos of the State of Mérida, Venezuela, at an altitude of 3,250 to 3,900 meters. Type collected by Linden (no. 1414).

This is the only species described from Venezuela which is not represented in Doctor Jahn's collections. Judging from the description it must be similar to the plant here described as *Espeletia bracteosa.* It is said to have heads twice as large, as well as leaves of somewhat different form. The heads are radiate, the rays being yellow.

15. **Espeletia bracteosa** sp. nov.

Basal leaves numerous, 20 to 25 cm. long, 3 to 4 cm. wide in the broadest part, the blades elliptic, acute or acutish at the apex, long-attenuate at the base to a stout margined petiole, this gradually dilated at the base, at first abundantly lanuginous on the upper surface, glabrous in age, very densely matted-tomentose beneath with fulvous hairs, with additional long loose white lanuginous hairs, the midvein prominent beneath, white-woolly, the lateral veins numerous, parallel, conspicuous beneath, visible also on the upper surface; bases of the petioles densely covered with long silky fulvous hairs; flowering sems 40 to 50 cm. high, bearing a few small, alternate, narrowly lanceolate or oblanceolate leaves below the inflorescence; inflorescence corymbosely branched, the branches erect or ascending, bearing numerous leaflike bracts 2 to 6 cm. long, these lanceolate, attenuate, pubescent like the leaves; branches of the inflorescence slender, densely lanuginous with brown hairs; peduncles 2 to 4 cm. long; heads rather few, about 12 mm. broad; involucral bracts oblong to linear, acute to attenuate, densely pilose with brown hairs; rays not seen; paleæ of the disk attenuate, densely covered above with short, stiff, appressed, viscid, reddish brown hairs; corolla tube densely viscid-pilose; achenes dark brown, smooth, sharply angled.

Type in the U. S. National Herbarium, no. 602350, collected in the Páramo de la Cristalina, State of Trujillo, Venezuela, at an altitude of 2,900 meters, December 20, 1910, by Dr. Alfredo Jahn (no. 156).

This appears to be most closely related to *Espeletia corymbosa*, but the leaves are shorter, relatively broader, and less acute, while the inflorescence is more open, more branched, and bears broader, less pubescent bracts. The corollas are densely pubescent, while for *E. corymbosa* they are figured as glabrous.

16. ESPELETIA CORYMBOSA Humb. & Bonpl. Pl. Aequin. 2: 13. *pl. 72.* 1809

Espeletia rigida Humb. & Bonpl. op. cit. *pl. 72*, nomen nudum.
Espeletia platylepis Schultz Bip.; Wedd. in Cast. Expéd. Amér. Sud Bot. 1: 64. 1855, as synonym.

TYPE LOCALITY: Vicinity of Almaguer, near the southern boundary of the State of Tolima, Colombia, at an altitude of 2,268 meters. Type collected by Humboldt and Bonpland.

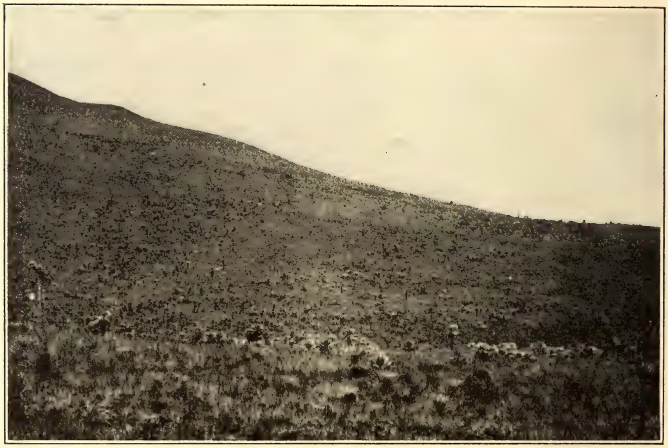
This species is apparently the southernmost representative of the genus. Weddell also refers here specimens collected by Goudot and by Linden (no. 1291) in the mountains near Bogotá. Judging from Bonpland's original illustration and description the plant is not far removed from some of the species here described as new from Venezuela.

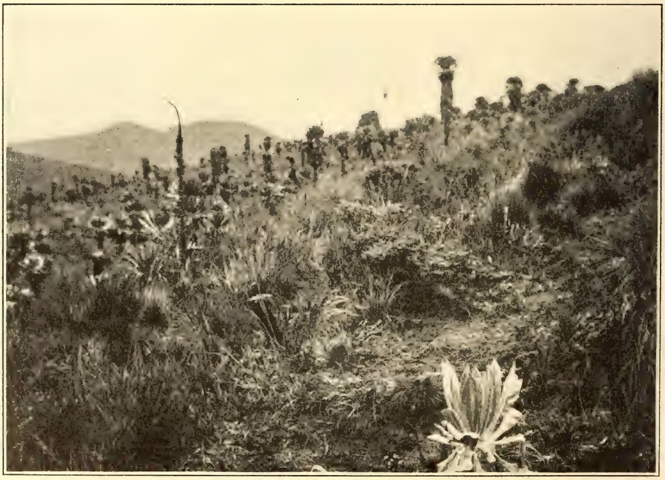
In the text of the original description the species is named *Espeletia corymbosa*, but the accompanying plate bears the name *Espeletia rigida*.

17. ESPELETIA FUNCKII Schultz Bip.; Wedd. in Cast. Expéd. Amér. Sud Bot. 1: 64. 1855

TYPE LOCALITY: Andes of Pamplona, Colombia, at an elevation of 3,400 meters. Type collected by Funck and Schlim (no. 1290).

The type locality is near the Venezuelan boundary and the species may be expected to occur in that country. Weddell compares this with *E. corymbosa*, saying that it is a larger plant with a less regular, more open inflorescence, and with more elongate achenes having sharper angles.





Espeletia grandiflora IN THE PÁRAMO DE BUENA VISTA, STATE OF CAUCA, COLOMBIA.
PHOTOS BY MR. H. PITTIER.

A STUDY OF THE RELATION OF TRANSPIRATION TO THE SIZE AND NUMBER OF STOMATA

Walter L. C. Muenscher

Considerable work has been done in determining the quantity of water lost from various plants under various conditions. Several extensive investigations have been conducted with a view of determining stomatal values for various species. Although several important researches have been conducted concerning the stomatal regulation of transpiration, very little has been done on the amount of transpiration in relation to the size and number of stomata per unit of transpiring surface. It was my aim to determine whether any relation exists between the amount of transpiration and the number of linear units of stomatal aperture per unit of leaf surface.

I. Historical

Probably the earliest attempt to determine quantitatively the amount of water lost in plants was by Hales (7) as early as 1727. Hales not only determined the amount of transpiration per square foot of leaf area for the sunflower, cabbage, grapevine, lemon tree, and apple tree, but he also found that the amount of transpiration varied during the day and night hours and also with changes in physical factors.

Clapp (4) determined the quantity of water in grams transpired (GM^2H) per square meter of leaf surface per hour for day and night for thirty common greenhouse plants both under greenhouse and under "standard" conditions, but she used only one individual of each species in her experiment. In computing the transpiring surface she considered only the upper surface of the leaf. She also found that there are two extremes of transpiration, the greatest amount in the early afternoon when the light is strongest and the minimum amount during the night when the stomata are closed.

The work on transpiration up to 1904 is summarized in Burgerstein's excellent work on transpiration in plants (3). He discusses the various phases of transpiration, methods for its determination, the effect of leaf structure and of external factors on transpiration, and whether transpiration is a process of vital significance to the plant or whether it is a necessary evil to the plant.

The work on the size and number of stomata for various species is rather limited but several rather extensive investigations have been conducted along this line of work. Several early investigators—Humboldt, 1786; Hedwig, 1793; Kieser, 1815; Lindley, 1832; Krocker, 1833; Meyer, 1837; Unger, 1855; and Morren, 1864—determined stomatal values for a number of different species, the results of which are given in a summary table by Weiss (1865).

Weiss in his very extensive work (13) gives the length, breadth, and area of stomata as well as the number of stomata per unit of area both on the upper and lower surface for 167 of the more common European plants. The results of these early investigators do not always agree and often their figures are very different. This may be explained as being due to the insufficient instruments for measuring, but more probably because different men used plants which were grown under different conditions or even used different varieties.

Weiss observed that stomata may be present on underground stems and aerial stems as well as on leaves. He also states as a result of his observations that the presence of stomata is not limited by the surrounding medium; in other words, stomata may be present on parts of plants which are in air, water, or earth.

Eckerson (5) determined the stomata-quantities for about 38 common greenhouse plants. She gives a valuable table in which are recorded the length of the guard cells and of the pore of the stomata and the number of stomata per sq. mm. of upper and lower leaf surface for each species.

The most important work on transpiration in relation to stomatal movement is by Lloyd (10). In his experiments on cuttings of *Verbena ciliata* and *Fouquieria splendens* he discovered that the rate of transpiration may undergo sudden and wide changes without any corresponding changes in the size of the stomatal aperture. From this he concludes that stomatal regulation does not occur, though, of course, conservation of contained water follows upon complete closure of the stomata; but it has not yet been proven that this ever occurs.

The plan of my work was to determine the quantity of transpiration simultaneously for a number of species with various stomatal values and then determine what relation, if any, exists between the amount of water lost and the amount of stomatal aperture in linear units, per unit of leaf area.

II. Method

Various methods have been devised and employed for the quantitative determination of transpiration both directly and indirectly. It seems that the results, in order to be of much value, should be obtained from plants which at least approach the natural conditions of the plant. Some investigators have confined their investigations entirely to observations upon removed parts of plants, small cuttings of branches, or even single leaves. Lloyd (10) worked only with cuttings in his experiments on stomatal movements. Freeman (6) questions the value of determinations of water loss made from detached parts of plants except where only comparative results are desired, which was the case in Lloyd's work (10). However, it is doubtful whether the limited number of experiments performed by Freeman (6)

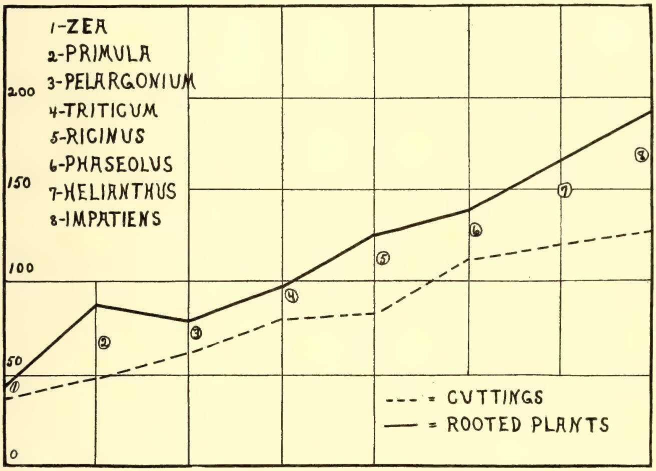


Fig. 1. Showing the amount of transpiration in mg. per hour per sq. dc. leaf surface for cuttings and rooted plants of the several species.

would warrant us in drawing the conclusion which he makes in regard to the relation of the amount of transpiration from rooted plants and detached cuttings of plants.

In order to satisfy myself as to the possibility of a relationship between the amount of water lost from plants with their roots and plant-parts without roots, an experiment was conducted with five cultures of cuttings in water and ten cultures of rooted plants in jars for each of eight species.

The two sets of jars were run simultaneously for 24 hours from 6 o'clock P. M. January 4, to 6 o'clock P. M. January 5, 1915. The results of the experiment are given in the form of curves in figure 1. The two curves show that the water loss in cuttings is about 20 or 30 percent less than the amount lost by the same plant under similar conditions when rooted in a jar. In every case the amount of transpiration was less per unit of leaf surface from cuttings than from rooted plants. The cuttings consisted of short stems with from two to five leaves and were removed from the plants under water. The quantity of transpiration is recorded in milligrams per hour per square decimeter of leaf surface.

In brief, the method employed in the following experiment consisted in sealing jars so as to prevent all loss of water except that which is lost through the plant; determining this loss by weighing; and then computing the amount of loss per unit of leaf area and stomatal aperture.

1. *Selection and Preparation of Plants*

In selecting the species used in this experiment several important considerations had to be kept in mind. Only plants with simple stomata were taken; that is, plants with stomatal elaborations such as pits, plugs, many hairs or extremely sunken stomata could not be used in this work so as to bring us face to face with the one factor, namely, the relation of the size and number of stomata to the amount of water lost.

The species had to be chosen so as to present the widest possible range in size of stomata. Only those plants could be selected which could be readily grown under greenhouse conditions such as those under which the experiment was performed: lilacs and cottonwoods were started, but shed their leaves and failed to send out a new set of normal leaves; plants of *Datura stramonium* were started from seed but the plants grew very slowly and were so much stunted they they could not be used in this work. Lastly only those plants could be used which would attain a fair size and maintain a reasonably uniform rate of growth while the experiment was performed. (See Table I.)

2. *Preparation of the Jars*

Most of the plants with the exception of *Pelargonium zonale* and *Primula sinensis* were started in flats from seeds. While the plants were still young they were carefully transplanted into the jars where they remained unsealed until they were large enough to use in the determination of transpiration. The plants of Primula and Pelargonium were healthy greenhouse plants which were transplanted directly into the jars and then were left on the greenhouse bench for several weeks before sealing for the experiment.

Large stone crocks of two sizes were used for containers; gallon crocks were used for the large plants, while one-half-gallon crocks were used for the smaller species. On the bottom of each jar was placed an inverted three-inch flower pot filled with gravel. Around the flower pot about one centimeter of sand was spread over the bottom of the jar. A glass tube with a 1 cm. bore was inserted into the hole in the bottom of the inverted flower pot and the tube extended about 10 cm. above the surface of the paraffin when the jar was sealed. This tube was used for watering the plant. The flower pot with gravel in it rapidly absorbed the water and the sand spread the water evenly over the bottom of the jar. Another tube was put in the jar so as to act as an aerating system for the soil when the jar was sealed. Then the soil was placed in the jars and the plants were planted in them. The number of individuals of each species that were placed in one jar varied from one to several, depending upon the size of the plants. (See Table I.) The cotyledons which still happened to be on the plants at this time were removed when they were transplanted.

After the plants had stood in the jars from one to several weeks, and had become well established they were ready to be sealed. The kind of seal used is that which was used by Briggs and Shantz (1) except that a little harder grade of paraffin was used. A mixture of eight parts of about 60° melting paraffin and two parts petrolatum was taken; however, the exact proportions were not determined in every case. The mixture was heated to melting and was then poured over the surface of the soil so that upon cooling it made a perfect seal.

In order to prevent the warm paraffin from doing any possible injury to the stem of the plants partially cooled strips of paraffin were first wrapped around each stem at the surface of the soil where the hot paraffin would otherwise come in contact with the stem. Each jar was marked by writing a number on the paraffin surface.

After the jars had been continued for several weeks the paraffin began to draw away from the edges of the jar slightly. In order to determine any possible error due to evaporation from the edge of the jar a number of jars were reweighed several times after the plants had been removed to see whether any water was lost by evaporation, but this was found to be very small or sometimes so small that it could not be detected by weighing from day to day. After removing the paraffin from the jars I found that it had run down between the edge of the soil and the stone crock several centimeters and still formed a very tight seal near the surface of the soil so that very little water could escape.

3. *The Determination of Water Loss*

A week after the jars had been sealed I took the first weighing for determining the amount of water lost. The amount of water lost was computed from the decrease in the weight of the jar between the intervals of weighing. At first the jars were weighed twice daily, between six and seven o'clock A. M. and between six and seven o'clock P. M. Later the weighings were taken only once each day in the evening, while some of the final weighings were taken at intervals of two or three days; but the jars were always weighed between six and seven o'clock P. M. For weighing I used a "micrometer scale" which has a capacity of 18,000 grams and weighs very accurately down to two or three grams.

The loss of water from the jar was replenished at intervals of two or three days through the glass tube which was inserted for that purpose. The amount of water added at one time was approximately the amount that was lost by the plants during the interval since the last addition of water, and was added in the evening between seven and nine o'clock.

4. *The Determination of Leaf Areas*

Numerous methods have been employed from time to time for the determination of leaf areas, but it was found impracticable to use any of these methods without modification. It was impossible to use the solio paper method which was employed by Sampson and Allen (12), for it necessitated the making of many thousands of leaf prints. I made a number of preliminary determinations of the leaf areas of a number of plants by comparative weights, that is by weighing a given area of leaves which was determined by a Ganong's leaf-area cutter and then weighing the entire leaf area of a plant, from this proportion I determined the total leaf area of the plant. Then I also determined the leaf area of the same plants by making leaf traces and then computing the area by a planimeter. I found that the planimeter method was not only the most accurate but also the most practicable method for this problem. I found that the weight of one square centimeter of leaf varied from 1 to 100 percent from the average weight, depending upon the part of the leaf from which it was taken. The loss of water from the cut leaf sections is also very great especially if the leaves are not weighed immediately. By the weighing method the leaf area had to be found at the same time in all the plants of one species because the amount of photosynthate in the leaf at any given time varies with the time of day as well as from day to day. This would necessitate the determination of the weight per unit area each time it was desired to determine the leaf area for a number of plants.

By the use of the planimeter one can find the leaf area of a plant regardless of the amount of water or photosynthate in the leaf and one can use the same method for almost any number or kind of plant at any time. But, because of the enormous number of leaves of which I had to find the area, it was found necessary to modify the method somewhat. I found that each species had a large number of leaves in each plant which were almost of the same size, so I sorted the leaves of each plant into five piles and measured the area of one leaf of each pile and then multiplied the area of one leaf by the total number of leaves in that particular pile and then I computed the total leaf surface for each plant in square centimeters, considering both the upper and lower surface of each leaf.

Petiolar and stem areas were not considered as transpiring surfaces. It was found that these organs were practically free from stomata and the water loss from the same was very small in the plants used in this experiment.

The leaf area was determined for each plant at the end of the experiment. Since most of the plants were small and had a small amount of leaf surface to begin with, compared with the leaf surface at the end of the experiment, the increase being due to growth, I had to make a correction for the increased leaf area. I considered the increase in transpiration as being proportional to the increase in leaf surface and divided the total leaf area by two to find the average leaf area during the entire period when the plants were used. This would be taking the leaf surface as zero to begin with but this discrepancy is balanced by the leaves which died and were removed from the plants during the progress of the experiment. This method was employed for all of the species which were started from seed. In the case of Pelargonium and Primula, two slow growers, where mature plants were used, I used the entire leaf surface as was determined at the close of the experiment. What little growth took place was offset by the loss of several dead leaves which were removed from each plant.

5. *The Determination of the Number of Stomata*

For determining the number of stomata I used a Spencer microscope with a micrometer scale. The value of the scale and of the field of the microscope were determined with a stage micrometer. The field was divided into quadrants by drawing very fine threads of balsam over the eyepiece micrometer. In those plants which had an epidermis which could be easily removed I mounted pieces of epidermis in absolute alcohol and then stained them with a weak solution of iodine. In the plants from which the epidermis could not be removed readily I counted the stomata "in situ" by the method suggested by Lloyd (9).

The stomatal counts were determined from an average of 30 to 50 sq. mm. each for the upper and lower surface from five or more leaves taken from as many different plants. Only fairly mature leaves were used and from these the fields were taken at random on the different parts of the surface.

6. *The Determination of the Size and Area of Stomata*

After determining the number of stomata for the several species the next process was to determine the size of the stomata for the same. In this process I employed the method so successfully used by Lloyd (10), Eckerson (5), Renner (11), and Livingston (8), in fixing the stomata so that they will not shrink and lose their shape and actual dimensions. This method is based upon the rapidity with which alcohol dehydrates the guard cells when bits of epidermis are dropped in absolute alcohol immediately after they are removed from the leaf. When once dehydrated these cells become hard and remain permanent in size and shape even if they are afterwards mounted in water.

Since Lloyd (10) and Eckerson (5) found that the stomata are open widest at about 10 o'clock A. M., I removed bits of epidermis at this time of the day on bright clear days, placed them in vials of absolute alcohol and then determined the length and breadth of both guard cells and pores for about 25 to 50 stomata for each leaf surface for the several species used. From these data I also computed the area of one stoma and the amount of stomatal aperture and the number of linear units of stomatal pore per unit of leaf area for upper and lower surface.

III. Experimental

The following tables with short explanations and discussions represent briefly the scope and results of this investigation.

Table I

Showing the Species and the Number of Individuals of Each Used and When Each Were Studied

Series	Species	Date	No. of Jars	No. of Plants in Each Jar	Total No. of Plants
1.	*Helianthus annuus* L.	11 days—Nov. 4–15, 1914	10	2	20
2.	*Helianthus annuus* L.	5 days—Dec. 20–24, 1914	10	4	40
1.	*Impatiens sultani* Hook.	30 days—Nov. 4–Dec. 4, 1914	12	2–3	30
2.	*Impatiens sultani* Hook.	25 days—Dec. 10, 1914–Jan. 3, 1915	6	3	18
1.	*Pelargonium zonale* Willd.	20 days—Nov. 4–24, 1914	10	1	10
2.	*Pelargonium zonale* Willd.	40 days—Dec. 10, 1914–Jan. 18, 1915	10	1	10
1.	*Phaseolus vulgaris* L.	25 days—Nov. 4–29, 1914	10	2–4	32
2.	*Phaseolus vulgaris* L.	40 days—Dec. 10, 1914–Jan. 18, 1915	10	4–6	48
1.	*Primula sinensis* Sabine	40 days—Nov. 30, 1914–Jan. 8, 1915	10	1	10
1.	*Ricinus communis* L.	60 days—Nov. 4, 1914–Jan. 4, 1915	10	2–3	21
1.	*Triticum sativum* Lam.	20 days—Jan. 3–23, 1915	8	10	100
1.	*Zea mays* L.	30 days—Nov. 4–Dec. 4, 1914	10	4	40
2.	*Zea mays* L.	30 days—Dec. 19, 1914–Jan. 18, 1915	10	4–5	45
	Total		128		424

Table I briefly shows the extent of the work performed. The first column of the table gives a list of the species used. The number before the name indicates the number of the series for that species. With several exceptions two series were performed for each species. The second column indicates for how many consecutive days the transpiration was determined for each series and also the date when the work was carried on. The third column gives the number of individuals in each jar and the last column contains the total number of plants used in this investigation.

TABLE II

Showing Average Amount of Transpiration from the Various Species Used in Series I

Species	Total Time in Days	Total Water Loss in Gm.	Total Leaf Area in Sq. Cm.	Average Transpiration in Mg. per Hour per Sq. Dc. Leaf Surface
Helianthus annuus	11	8,027	20,000	151
Impatiens sultani	30	10,862	6,200	243
Pelargonium zonale	20	8,930	38,000	50
Phaseolus vulgaris	25	12,440	13,400	155
Ricinus communis	60	25,897	8,600	209
Zea mays	30	23,047	41,000	78

TABLE III

Showing Average Amount of Transpiration from the Various Species Used in Series II

Species	Total Time in Days	Total Water Loss in Gm.	Total Leaf Area in Sq. Cm.	Average Transpiration in Mg. per Hour per Sq. Dc. Leaf Surface
Helianthus annuus	5	1,440	7,200	166
Impatiens sultani	25	5,805	3,800	255
Pelargonium zonale	40	11,610	18,600	65
Phaseolus vulgaris	40	15,200	10,100	156
Primula sinensis	40	6,580	9,200	75
Triticum sativum	20	1,900	5,100	79
Zea mays	30	4,700	8,024	82

Upon examining Tables II and III it will be found that the absolute amount of water lost for the various species varies very much because of the variation in the total amount of leaf surface in the several species. It will also be noted that the average quantity of water lost per square decimeter of leaf surface, average of upper and lower surface, varies from a minimum of 50 mg. in Pelargonium to a maximum of 235 mg. in Impatiens per hour, average of day and night transpiration for the average of ten jars for about 40 days.

The third column of Table II shows the total amount of water lost in grams by all the jars of each species used in series I. Column four shows the total, upper and lower, leaf surface in square centimeters for each species used in series I. Column five shows the average amount of water transpired for each square decimeter of leaf surface per hour for each species. Table III records the same data for the second series (Series II).

A comparison of the results of the two tables (Tables (II and III shows that the average amount of water transpired by each species as determined in the two series of plants varies but slightly. The largest difference is found in *Pelargonium zonale* and this is well accounted for by the fact that I accidentally used a different variety of Pelargonium for the second series. The results of *Helianthus annuus* may have been modified by an early attack of *Erysiphe cichoracearum*, which prevented me from continuing the cultures for a longer period than five days in the second series. The first eleven days of weighings which were obtained from the first series were taken before there was any evidence of Erysiphe and, I think, represent normal transpiration.

TABLE IV

Showing the Minimum, Mean, Average, and Maximum Number of Stomata in One Square Millimeter Leaf Surface

Name of Species	Lower Surface				Upper Surface			
	Min.	Mean	Av.	Max.	Min.	Mean	Av.	Max.
Phaseolus vulgaris	131	269	250	327	28	40	40	50
Ricinus cummunis	79	156	121	172	35	68	52	76
Zea mays	91	103	101	110	51	61	60	83
Primula sinensis	49	87	84	112		0		
Pelargonium zonale 1	32	52	52	72	9	18	19	24
Pelargonium zonale 2	190	210	215	232	4	9	8	11
Impatiens sultani	105	140	143	180	12	37	29	70
Triticum sativum	12	21	21	27	35	46	46	55
Helianthus annuus	125	170	172	198	27	70	71	90

Table IV shows the number of stomata per square millimeter leaf surface for each of the several species. It will be noticed that all but one species, *Triticum sativum*, have fewer stomata on the upper surface than on the lower surface. I found that the number of stomata varied considerably in different parts of the same leaf. On the other hand, I noticed that for similar areas of the various leaves examined the number of stomata was more or less constant for the species. I tried to take the upper and lower counts for each leaf from similar or corresponding areas.

The number of stomata per square millimeter varies from an average of a maximum of 250 in *Phaseolus vulgaris* to a minimum of 21 in *Triticum sativum* for the lower surface and from a maximum of 71 in *Helianthus annuus* to a minimum of zero in *Primula sinensis* on the upper leaf surface. (See Table IV.)

In Table IV I recorded the minimum, mean, average, and maximum number of stomata determined per square millimeter for each species. The average number in each case represents the average of thirty or more single counts. The minimum and maximum are rather widely separated but the mean and average are nearly always equal or nearly so.

These figures do not compare exactly with any figures found for the same species by Weiss (13), or Eckerson (5), but the differences are not so large but what they may be accounted for by differences in conditions under which the plants were grown, or different varieties or strains might have been employed by the different investigators.

In *Impatiens sultani* I found 29 stomata per square millimeter on the upper leaf surface while Eckerson reported that no stomata were found by her on the upper leaf surface of the same species. Probably I used a different variety of *Impatiens sultani*. The table also shows two sets of figures for *Pelargonium zonale*, number I was used in the first series and number II was used in the second series. These are two different varieties, the former has fewer and much larger stomata than the latter variety.

Table V records the size of the stomata for the upper and lower surface for the various species. The length and breadth of the guard cells and of the pore is recorded in microns. Each number represents the average of thirty or more measurements. I found that all the stomata as well as their pores were more or less elliptical. The length of the pore is about one half of the total length of the guard cell apparatus. (See Table V.) The width of the pore is usually less than one half its length. The largest stomata were found on the upper epidermis of *Triticum sativum*, with a pore 39 microns in length; the smallest stomata were found on the upper side of *Impatiens sultani* with a pore six microns in length. The stomata which were measured were taken from several pieces of epidermis from several leaves from at least three plants. It was noted that individual stomata of the same plant or even leaf vary somewhat in size depending upon the part of the leaf in which they are located.

TABLE V

Showing the Length and Breadth of Stomata in Microns

Name of Species	Lower Surface		Upper Surface	
	Guard Cell	Pore	Guard Cell	Pore
Phaseolus vulgaris	20 × 15	8 × 3	20 × 13	11 × 3
Ricinus communis	26 × 18	10 × 3	22 × 15	10 × 3
Zea mays	36 × 27	19 × 3	33 × 19	19 × 3
Primula sinensis	42 × 31	20 × 4	——	——
Pelargonium zonale 1	38 × 27	20 × 4	38 × 27	17 × 3
Pelargonium zonale 2	28 × 18	11 × 3	30 × 21	12 × 5
Impatiens sultani	20 × 16	9 × 3	17 × 14	6 × 3
Triticum sativum	62 × 30	34 × 3	65 × 29	39 × 3
Helianthus annuus	37 × 26	19 × 4	30 × 24	14 × 3

Upon comparing the results of Tables IV and V, I noticed several general facts:

1. The size of stomata on the same plant may vary considerably with the upper and lower leaf surface.
2. In general plants with few stomata have large ones and plants with many stomata have small ones.

From the data in Tables IV and V, I computed the average number of units of stomatal pore length in microns per square millimeters of leaf area for the upper leaf surface and for the lower leaf surface by multiplying the number of stomata per unit of area by the average length of one pore. Then I computed the average number of microns of stomatal pore length for the upper and lower surfaces by adding the two values and dividing by two. The linear units of stomatal pore and not the area of the stomatal pore is important since Brown and Escombe (2) have shown that the length of the pore and not its area is important in transpiration.

I also computed the area of the stomatal pore of one stoma and then the area of the total amount of stomatal aperture per square millimeter, average of upper and lower leaf surface, by considering the stomatal pore an ellipse. (See Table VI.)

The data show that it is not necessarily the plant with the largest stomata or the plant with the greatest number of stomata that has the largest area of stomatal aperture per unit of leaf surface. The largest number of linear units of stomatal pore, average of upper and lower surface, was found in *Helianthus annuus*, 2056 microns, and in *Zea mays*, 1530 microns, while the smallest number was found in *Pelargonium zonale*, 682 microns, and in *Impatiens sultani*, 731 microns. (See Table VI.)

TABLE VI

Showing the Number and Size of Stomata, and the Linear Units of Stomatal Pore in Microns per Square Millimeter Leaf Surface

Species	Average No. of Stomata per Sq. Mm. Surface		Average Length of Pore in Microns		Average No. of Linear Units of Stomatal Pore
	Lower	Upper	Lower	Upper	Average of Upper and Lower Surface in Microns per Sq. Mm.
Phaseolus	250	40	8	11	1,220
Ricinus	121	52	10	10	865
Zea	101	60	19	19	1,530
Primula	84	0	20	—	840
Pelargonium 1	52	19	20	17	682
Pelargonium 2	215	8	11	12	1,230
Impatiens	143	29	9	6	731
Triticum	21	46	34	39	1,254
Helianthus	172	71	19	14	2,056

I now arranged the several species in Table VII in the order of their greatest number of linear units of stomatal pore and also placed opposite each species in the third column the average amount of water transpired per square decimeter per hour in milligrams. This average amount of transpiration was obtained from the data in Tables II and III.

TABLE VII

Showing Relation Between the Amount of Transpiration and Stomatal Aperture

	Linear Units of Stomatal Pore in Microns per Sq. Mm.	Amount of Transpiration per Sq. Dm. per Hour in Mg.	Area of Stomatal Aperture in Sq. Microns per Sq. Mm. of Leaf Surface
Helianthus annuus	2,056	156	6,433
Zea mays	1,530	80	3,864
Triticum sativum	1,254	79	2,998
Pelargonium zonale 2	1,230	65	2,868
Phaseolus vulgaris	1,220	156	2,890
Ricinus communis	865	209	1,989
Primula sinensis	840	75	2,614
Impatiens sultani	731	249	1,705
Pelargonium zonale 1	682	50	1,992

From this table (Table VII) I could determine whether any relation exists between the amount of water lost and the amount of linear units of stomatal pore. These results do not show any constant relation between the amount of transpiration and the number of linear units of stomatal pore. The two species, *Impatiens sultani* and *Ricinus communis*, both of which have a small amount of stomatal pore per unit of leaf surface, have the two highest amounts of transpiration per unit of leaf surface. *Phaseolus vulgaris* also has a high transpiration in proportion to the amount of stomatal pore per unit of leaf surface.

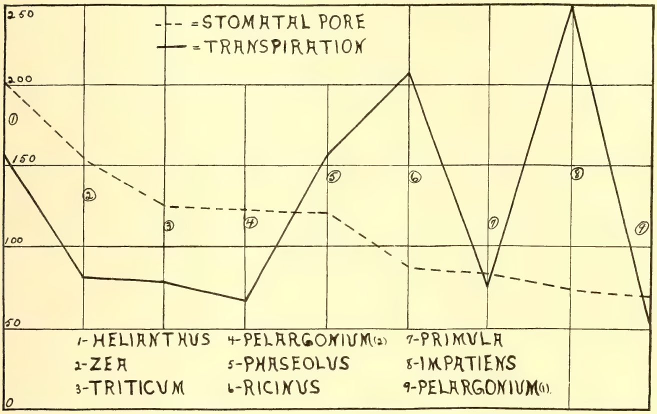


FIG. 2. Showing the amount of transpiration in mg. per hour per sq. dc. leaf surface and the number of linear units of stomatal pore in microns per sq. mm. leaf surface.

The data of Table VII are also shown in graphic form by two curves in figure 2. The two curves are drawn to separate scales. In the transpiration curve the side of each square represents fifty milligrams. In the curve showing stomatal pore the side of each square equals 500 microns. In other words the latter curve represents values ten times as large as the former, or each unit equals ten microns in the curve which represents stomatal pore.

In Table VIII I have arranged the species in the order of the

TABLE VIII

Showing Length, Area, and Amount of Transpiration for One Stoma

	Ave. No. of Stomata per Sq. Mm.	Ave. Length of Stomatal Pore in Microns	Ave. Area of 1 Stoma in Sq. Microns	Ave. Transpiration of 1 Stoma per Hour in Grams
Phaseolus vulgaris	145	9.5	22	.00010
Helianthus annuus	122	16.5	47	.00013
Pelargonium zonale 2	112	11.5	35	.00006
Ricinus communis	87	10	23	.00022
Impatiens sultani	86	7.5	18	.00029
Zea mays	81	19	48	.00009
Primula sinensis	42	20	62	.00018
Pelargonium zonale 1	36	18.5	51	.00014
Triticum sativum	34	36.5	85	.00023

largest number of stomata, the average of the upper and lower surface. I also recorded the average length of the pore of one stoma in microns, the area of one stoma in square microns, and the amount of transpiration from one stoma in hundredths of a milligram.

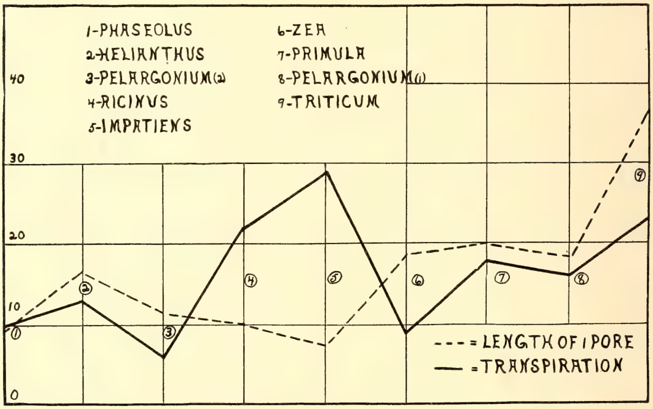


FIG. 3. Showing the length of one pore in microns, and the amount of transpiration in hundredths of mg. per hour for one stoma in the several species.

The data, namely the length of the stomatal pore and the amount of transpiration per stoma for the various species, are represented in graphic form by two curves in figure 3. These figures do not show any relation between the length or area of the pore of a stoma and the amount of water lost for the several species.

SUMMARY

1. There was found no constant relation between the amount of water lost and the numbers of linear units of stomatal pore, *i. e.*, the number of stomata per unit of leaf surface multiplied by the length of the average pore, in the various species studied.

2. There is no relation between the amount of transpiration and the length of the pore of one stoma. The number of stomata per unit of leaf surface however varies at the same time that the length of the pore varies for the several species; so in this case we have two variables.

3. There is no relation between the amount of transpiration and the number of stomata per unit of leaf surface in the different species investigated.

4. From the above results it would seem that the amount of transpiration is not governed entirely by stomatal regulation, and that the variations in the amount of water loss in different species cannot be accounted for by the size and number of stomata but must be explained perhaps by a complex of several factors.

This investigation was suggested by and conducted under the direction of Professor Raymond J. Pool, of whose kindly advice and suggestions as well as the encouragement and suggestions of Dr. C. E. Bessey, I wish to express my sincere appreciation.

UNIVERSITY OF NEBRASKA.

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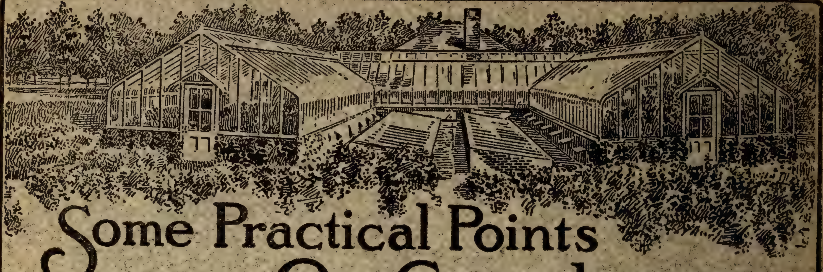

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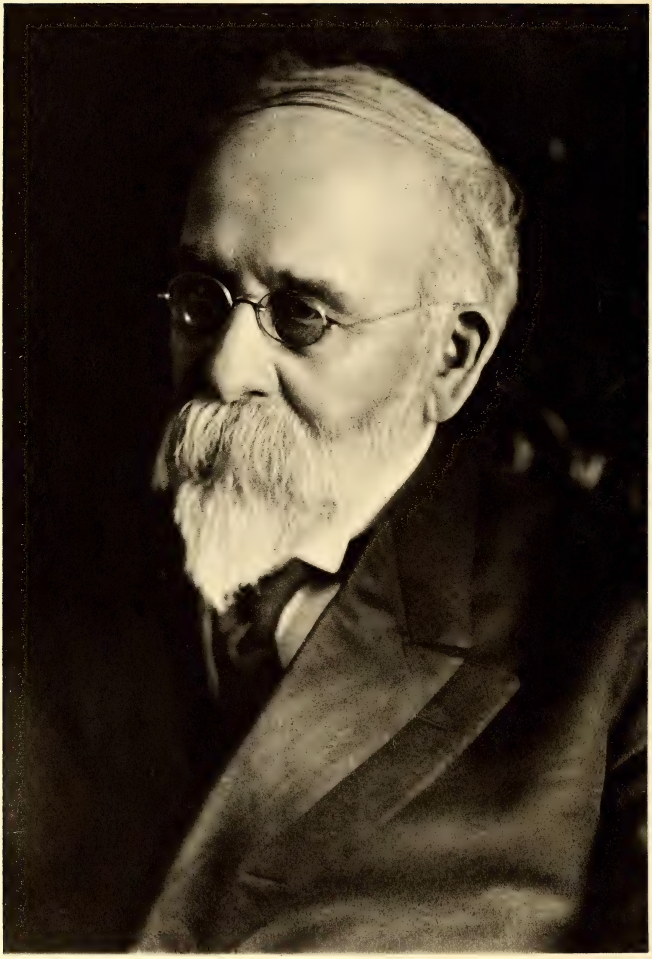

Charles E. Bessey

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A BRIEF SKETCH OF THE LIFE AND WORK OF CHARLES EDWIN BESSEY

(WITH PORTRAIT)

RAYMOND J. POOL

Charles Edwin Bessey, professor of botany and head of the department of botany in the University of Nebraska since 1884 and a conspicuous figure in American science and education, passed away at his home in Lincoln on February 25, 1915, after a critical illness of four weeks.

The Bessey family is of French extraction, the original form of the name being Bessé. The tradition is that the early members of the family, who were Huguenots, were compelled on account of religious persecution to flee to England from the old home near Strassburg in Alsace. This exodus occurred in the latter part of the seventeenth century after which the "y" was added to the name.

The Besseys remained in England for several generations. Among the first of the family to come to America was Jacob Bessey who, about the middle of the eighteenth century, emigrated from England.

Jacob Bessey married and settled near Doylestown, Pennsylvania. One of the sons of this union was Michael, who was a weaver by trade. This son married Mary Wismar, a descendant of a family from the Palatinate on the Rhine. Michael and Mary Bessey were the parents of Adnah Bessey who was born in eastern Pennsylvania in 1812. In 1832 Adnah Bessey's family with many others from the vicinity of his home migrated westward and settled in Wayne County, Ohio. Adnah Bessey married Margaret Ellenberger. Emmanuel Ellenberger, the father, died when Margaret was but a young girl and the widowed mother married again. Margaret's stepfather was Jacob Kimmel, a widower with a large family. Margaret was accordingly

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"put out to live" with James McKinly and wife, an estimable couple who had also migrated from Pennsylvania to Ohio. This young lady was sent by her foster parents to a school which happened to be taught by Adnah Bessey, and some time later (1841) teacher and pupil were married. Adnah Bessey and Margaret Ellenberger Bessey were the parents of Charles Edwin Bessey, who was born in a log house on a farm in Milton Township, Wayne County, Ohio, May 21, 1845. Much of the early education of Charles, the boy, was received under the direct attention and supervision of Adnah, the father. In his seventeenth year he received a certificate to teach school. He did not teach at that time, however, as he had made all arrangements to enter the academy at Seville (Ohio) in order to fit himself for college. His educational training was interrupted soon after this by his father's ill health, but even then he attended the district school and studied algebra by himself. His father died in 1863 and that fall Charles attended the academy for five weeks. He then began teaching at Wadsworth, Ohio, where he remained for a term of four months. Following this (March, 1864) he again entered the academy where he studied for two months, when the breaking up of the school precipitated another halt in the young man's educational career.

After several years of broken but persistent preparation in the country school and academy young Bessey entered the freshman class of Michigan Agricultural College in July, 1866. He completed his college course at the age of twenty-four and graduated from the college with the degree of bachelor of science, November 10, 1869.

When Bessey entered the college he firmly intended to return home to follow the profession of civil engineering or surveying. But he came to love the plants of the fields and forests. Finally his marked attention to such things became noticeable to others; for, after two years or so in college, the President (Abbott) and some of the professors (especially Prentiss) advised him to specialize in botany. At first the advice was rejected. But after weeks of reflection the young man found that his inclination had changed from engineering to botany.

Immediately after graduation Bessey was awarded an assistantship in horticulture and was placed in charge of the greenhouse at the Michigan Agricultural College. This position was held for a very short time because in December of that year (1869) the offer of an instructorship in botany and horticulture came from the Iowa State

College of Agriculture at Ames. The call was accepted and he began his work at Ames, in February, 1870. An interesting point of historical note is seen in the fact that he attended and took part in the first Farmers' Institute held in Iowa; this was during the winter of 1870–1871. These were among the very first meetings of the kind ever held in any part of the United States. It also appears that he was the guiding spirit in the founding of the old (the first) Iowa Academy of Sciences. He was president of this organization in 1875 and was re-elected regularly for several years.

Bessey was elected to membership in the American Association for the Advancement of Science at the Dubuque, Iowa, meeting in August, 1872. This was the first occasion of his meeting with Dr. Asa Gray, who was then the retiring president of the Association. Here he also met Dr. How, Dr. Winchell, M. S. Bebb, and others who were to be some of his closest scientific friends and co-laborers for many years. The first fruits of this acquaintance with Gray were gathered during that winter of 1872–1873 when he spent the long vacation of three months at Harvard University studying under Professor Gray. Under the watchful eye and kindly direction of the great botanist he laid the foundations of his knowledge of systematic botany especially in its philosophical aspects. The genial physician led the young man beyond the threshold of a "life among species and phyla" which was probably Dr. Bessey's most constant scientific enjoyment. Bessey returned for another period of study at Harvard in the winter of 1875–1876.

In 1872 he received the degree of master of science from his alma mater and was promoted to a full professorship at Ames. In November, 1873, he was offered the chair of botany, zoology, horticulture and pomology but this was refused. Later he was made professor of botany and zoology.

Early in the year 1874, at the suggestion of Dr. Gray, President Gilman, of the University of California, wrote to Professor Bessey asking him to give his views in regard to certain problems relating to agricultural education then demanding solution. His reply, along with those from Louis Agassiz, Andrew D. White, Samuel W. Johnson and others, was printed in a bulletin issued in the spring of 1874 by the University of California under the title: "Recent Information respecting Agricultural Education Elsewhere." This correspondence led to an invitation from President Gilman to Professor Bessey to go to California to give a series of botanical lectures in the university the following winter. The invitation was accepted and resulted in the appointment of Professor Bessey as a temporary "Lecturer on Botany."

In 1879 the University of Iowa conferred the degree of doctor of philosophy upon Professor Bessey in recognition of his publications in botany and as a partial reward for what he had already accomplished for the state of Iowa.

After having served the state of Iowa and its Agricultural College faithfully and efficiently for a period of fifteen years there came in June, 1884, notification that he had been elected professor of botany in the University of Nebraska. He went to Lincoln but found nothing in botany at the institution that had called him, and he was reluctant to leave the accumulation of his labors at Ames to go to a new state to build up another department from the very beginning. So he declined. A second offer was made in August of the same year. After another trip to Lincoln and a consultation with the regents this position was accepted and his inaugural address was delivered in September, 1884.

In Nebraska he began at once to collect data with reference to Nebraska grasses and the other plants of the state, and to make addresses. With the late Governor Furnas he organized the first series of Farmers' Institutes, which thereafter were periodically enlivened by his presence.

Bessey was a power among his fellow scientists, who were to be found in all parts of the world. From the time he first met Gray at Dubuque he seldom missed one of the annual gatherings, and he always took a keen interest in the administration of the various societies and in the scientific programs. The esteem of his associates was often reflected by the offices to which he was elected. In 1872 he became a member of the American Association and in 1880 was elected a fellow in the same. In 1889 he was president of the Society for the Promotion of Agricultural Science, and also of the Western Society of Naturalists. He was vice-president of the American Association and chairman of Section G (botany) in 1893, 1894, 1902, and 1907. He was also a charter member of the Botanical Society of America, of which he was president in 1895. That same year he was also president of the department of science of the National Educational Association. He was chosen to be botanical editor of the American Naturalist in 1880, a position which he held until 1897 when on invitation he accepted a similar position on the staff of Science.

The crowning scientific honor came at the Minneapolis meeting of the American Association for the Advancement of Science in 1910–1911, when he was elected president. The following winter (1911–1912) at Washington, D. C., he presided over the deliberations of this organization. When he returned to Lincoln after the meetings he was given an ovation by the students, faculty and the regents in the university chapel. The next winter (1912–1913) at the Cleveland meeting of the Association Dr. Bessey gave his address as retiring president. The paper which he read at that time was entitled: "Some of the Next Steps in Botanical Science."

Bessey was one of the pioneers who did much to lay the foundations of the present superstructure of American botany. The training with Gray naturally increased his love for taxonomy which is reflected in many of his publications. But he was thinking of other phases of botany also and some of his early papers show that he took advanced ground as to what should constitute the content of botany. In his paper on "The Diseases of Plants" (1882) we have abundant evidence that he was doing more than recording species and making herbaria. At this time the heterecious nature of rusts was not accepted by all botanists as conclusively demonstrated but Bessey felt that De Bary's conclusions were correct. The comments upon bacteria as disease-producing organisms are also interesting.

When in the early eighties the United States Department of Agriculture was considering the proposition to establish federal aid for state agricultural experiment stations he was consulted in regard to the wording of a bill for that purpose. He plunged into this work with the usual readiness and vigor and he finally wrote the paragraph defining the duties of such experiment stations verbatim as it was adopted later and became a part of the law known as the Hatch Act. He also wrote the first and second annual reports of the Agricultural Experiment Station of Nebraska in 1888 and 1889, and from that time he did not cease to concern himself actively with the progress of these institutions which were to become important seats of learning and research throughout the country.

Much of the time that Dr. Bessey gave to study and writing was devoted to a painstaking survey of the structure and evolution of all of the main groups of the plant world. This monumental work was begun many years ago, the first paper in the series dealing in particular with the higher plants being entitled: "The Phylogeny and Taxonomy of the Angiosperms" which was read as the address of the retiring president of the Botanical Society of America at its third annual meeting at Toronto, Canada, August 17, 1897. This paper contains a further statement of the thought and principles of a still earlier production—"Evolution and Classification"—which was given as the vice-presidential address of the chairman of the botanical section of the American Association at the Madison, Wisconsin, meeting in 1893. For a quarter of a century Professor Bessey worked upon the "phyletic idea" in taxonomy, the above papers being among the earlier ones upon that subject. His last paper entitled: "The Phylogenetic Taxonomy of Flowering Plants" was one of a memorable series of papers read by invitation at the Twenty-fifth Anniversary Celebration of the Missouri Botanical Garden, at St. Louis, October 15, 1914. The gathering at St. Louis at that time was his last meeting with the botanists of America.

But after all has been said about all of the other features of this great man's life we still must conclude that the most powerful and far-reaching effects of his captivating magnetism were recorded in the classroom, in the laboratory, in the college and in the university as a teacher and guide for the young. Professor Bessey utilized this potential to the limit, for he was one of the greatest teachers that the world has known. His powerful presentation of subject matter in the classroom was magnified by a personality which, because of its quaint paternal cordiality, won the admiration of thousands of students.

His lectures, delivered with a heavy clear voice and in non-technical phraseology, were always illustrated by means of quickly executed blackboard sketches which served at once to portray morphological features graphically and to drive home to the students' understanding exactly what he was talking about. He was so skilled with this method that he seldom used charts or diagrams prepared before class time. His small alarm clock and the black cloth-covered record book with pencil attached were inseparable adjuncts to every lecture.

Among the many services Professor Bessey rendered to the teaching of botanical science that might be mentioned was the introduction in 1873 at Iowa Agricultural College of the laboratory method of instruction with the use of compound microscopes. It is said that the laboratory method for advanced students was introduced at Harvard the year before, but this was wholly unknown to Bessey.

During the month of July, 1881, he gave the first course in botany in which laboratory work was offered at the University of Minnesota. He used to tell with much pleasure of reminiscence how he "carried the first compound microscopes to Minnesota." These instruments were borrowed from the college at Ames for that particular session, since the University of Minnesota possessed no such microscopes at that time. There were none at Ames when he went there. Neither were there any at Nebraska when he went there.

Bessey's students were numbered by the thousand and one of his greatest pleasures was to look over the lists of former students of his department and to picture them, oftentimes in distant lands, contributing of their thought and life for the betterment of mankind. Dozens of instructors in American schools and many investigators in the offices and laboratories of many institutions of learning and research owe their very life-ambition to the initial boost administered by Dr. Bessey at some critical moment. This was true not only of the botanically inclined but also of others whose primary inclination had drawn them into other fields. After a careful estimate made a few months before his death Professor Bessey came to the conclusion that in his forty-five years as a college professor he had had over 4,000 students in his classes. This was the work closest to his heart and in this he felt that he had fulfilled a divine commission. The love for young people and for the work of the teacher kept him young in spirit and vigorous in action to the end.

A large factor in moulding Bessey's standing as a botanist and in bringing him success as an educator was his series of text-books. In 1878 he began the preparation of a work on "Botany for High Schools and Colleges." The publishers had asked Dr. Gray to write this book, but he declined and recommended Professor Bessey. The manuscript was finished in January, 1880, and the book appeared August 1 of that year. This text went through two later editions, one in 1881 and another in 1883.

Then followed "The Essentials of Botany," the briefer course, of 1884. This book, which went through seven editions, the last being in 1896, enjoyed the enviable reputation as one of the most popular and most widely used texts in America. His last book, "The Essentials of College Botany" which he wrote (1914) with the assistance of his son, Dr. Ernst A. Bessey, was really another (the eighth) edition of the first elementary book.

At Nebraska, Bessey's ability as a college administrator was recognized at various times by the regents. At one time he was also acting president at Ames. He went to Nebraska as professor of botany and dean of the Industrial College. This deanship he held from 1884 to 1888 and again from 1895 to 1909, when he was made head dean. He was dean of the college of literature, science, and the arts from 1888 to 1891. He was made acting chancellor of the university in the summer of 1888, being called home from Europe, where he had gone with Mrs. Bessey to spend the summer in study. This position he held until 1891 when he succeeded in obtaining the appointment of Professor Canfield as chancellor. He was made chancellor *ad interim* again in 1899, and still once more, in 1907.

On December 25, 1873, Charles E. Bessey was married to Miss Lucy Athearn of West Tisbury, Martha's Vineyard, Massachusetts. To them three sons were born: Edward, Ernst, and Carl. All of the sons graduated from the University of Nebraska. Edward and Carl specialized in electrical engineering. The former was assistant professor of electrical engineering in the Colorado Agricultural College at Fort Collins at the time of his death on July 12, 1910. Carl is at present assistant chief engineer of the firm of Byllesby and Co., Chicago. Ernst specialized in botany and is at the present time professor of botany at the Michigan Agricultural College, his illustrious father's alma mater. Mrs. Bessey retains her home at 1507 R Street, Lincoln.

THE UNIVERSITY OF NEBRASKA, LINCOLN.

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[1] I wish to thank Dr. Ernst A. Bessey for help in connection with the preparation of this sketch, also Professor T. J. Fitzpatrick for a number of titles that have been included in the bibliography.—R. J. P.

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HEREDITY AND MUTATION AS CELL PHENOMENA*

R. Ruggles Gates

Heredity is frequently defined as the tendency of like to beget like; and the degree of resemblance between parent and offspring is often considered to be a measure of inheritance. The modern Mendelian work with dominant and recessive characters has, however, rendered such definitions incomplete and therefore untenable, since a plant or an animal may inherit in a predictable manner characters which its immediate ancestors did not exhibit at all. Two white races of sweet pea may on crossing give rise to a purple race, a reversion to an ancestral type which in the absence of definite knowledge might have been looked upon as an unexpected variation. But breeding experiments with the two white races will show that their germinal constitution is different. The interpretation then follows that the purple character is not a variation but is a result of inheritance. What has been inherited, however, is not a similarity but a difference.

In similar fashion the French zoologist Cuénot found that in certain cases where a wild gray mouse is crossed with an albino the second generation of offspring contain not only the two original types but black as well, the frequency being 9 gray: 3 black: 4 albino. Breeding experiments with the albino parent disclosed the fact that some of the germ cells of the albino carried the potentiality of producing black under certain conditions, *i. e.*, when meeting a germ cell containing the capacity or factor for developing color. Again we are dealing with phenomena of inheritance not of similarities but of differences. In the same way, in all sexually reproduced organisms it is not the similarities but the differences between the ancestors, or between the offspring, that we remark upon as being inherited. We can only speak of a boy inheriting his father's shape of nose or his mother's color of eyes when his parents differ in these attributes.

It has therefore become necessary to reverse in a sense the usual point of view with regard to heredity. Since it is inaccurate to say that heredity is measured by the degree of resemblance between parent and offspring, we may state that heredity consists in the perpetuation of the *differences* between related organisms. Inheritance is then the process by which these differences are perpetuated from generation to generation. This manner of statement is particularly useful when we contrast heredity and variation from the evolutionary point of view. For we may then define variation as the process by which new differences arise, and inheritance as the process by which they are perpetuated.

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These definitions have much more biological usefulness than may at first appear. They enable us to compare the phenomena of heredity and variation from a different point of view.

Though the above definition of variation applies to all kinds of variations, yet it has in view particularly mutations, which are completely inherited. We may classify variations as regards their heritability, into three classes: (1) those which are completely inherited, (2) those which are non-inherited, (3) those which are partially inherited. These three classes of variations must then have very different evolutionary significance. It is obvious that completely inherited variations, or mutations, are immediately effective for evolution or at least for species-formation, though it does not necessarily follow that they are of greatest evolutionary significance. It is not necessary to enter into this question here, nor discuss the relative importance of classes (1) and (3),—matters which are still to a large extent in dispute. We may, however, point out that bathmic variations, such as the rectigradations of Osborn, may come in a different category still as regards their relation to evolutionary processes.

The relative evolutionary significance of mutations and continuous or partially-inherited variations can be determined in part by comparison of specific differences in particular groups with the mutations which occur in those groups. Although a mere beginning has as yet been made in this direction, yet it would appear that mutations have probably played a larger part in specific differentiation in some groups than in others. But it will be a long time before these questions can be definitely decided. We may, however, affirm without doubt that in many groups mutations have played an important and even a preponderant part in species formation. Therefore whatever place may ultimately be assigned to mutation in the hierarchy of evolutionary factors in relation to the paleontological history of organisms, it has undoubtedly played an important rôle in the process of speciation.

Considering now certain features of mutations as we know them, one of the most interesting is their variety. Many attempts have been made to explain all mutations in terms of one idea. These universal explanations have involved (1) redistribution of Mendelian characters, (2) loss of factors, (3) reduplication of gametes, and many other hypotheses. They have nearly all, however, involved the idea of the mere loss of qualities or the recombination of those already existing. Such views have been brilliantly advocated, particularly by Mendelian writers. But they consider the phenomena of mutation largely from the outside. The cytological and anatomical combined with the experimental investigation of particular mutations already reveals that mutations belong in various categories and are the result of different types of change.[1]

Germinal changes are not due merely to plus or minus variations, in the loss or addition of Mendelian factors; but on the other hand each change results from a morphological or physiological alteration affecting one element of the germ plasm. This being the case, there is plenty of material at hand for progressive and divergent evolution, and it is unnecessary to imagine that the endless variety of organic structure has resulted from successive germinal simplifications by means of loss.

In a brief analysis of the various known types of germinal change, they may be classified into (1) those which are fundamentally morphological and (2) those which are primarily chemical. It may be expected in general that these two classes of changes will frequently be inherited in different ways. This brings us to the question of the relation between heredity and particular variations in another aspect. We have already observed that variation has to do with the *origin* of differences between organisms, and heredity with the *perpetuation* of those differences. It may here be pointed out that in the inheritance of any character-difference such as we are considering, there are two features to be taken into account; (1) the nature of the character itself, and (2) the mechanism of its inheritance. It is true that in Mendelian inheritance the mechanism by which the germinal determiners of the various character-differences are distributed in the germ cells during meiosis is the chief feature to be considered. But since it is now clear that in certain cases these characters are diluted and otherwise modified by crossing, the nature of the character and the possibility of its modification through hybridization can never be wholly neglected. In certain cases this feature of change in the character becomes of great importance, overshadowing the mechanism by which the fundament of the character is transmitted. I am, of course, speaking here of permanent modifications in a character, and not merely of the temporary effects which may be produced in a heterozygous organism through the presence of other factors.

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[1] For a discussion of this subject see Gates, The Mutation Factor in Evolution, Chap. IX. MacMillans: London. 1915.

When we study the nature of mutations which are fundamentally morphological it first becomes evident that each mutation is essentially a cell change, transmitted as such to every cell of the organism through mitosis. Before considering this aspect of mutation let us classify some of the known types of morphological mutations. For this purpose we will consider chiefly the genus Oenothera where these types of change have been most fully studied. It seems clear that all or nearly all the changes to which I refer have originally taken place in the nucleus of the cell.

Referring first to chromosome changes, in the genus Oenothera the original number of chromosomes is 14. This is true of *Oe. Lamarckiana* and many other species. Duplication of one of these chromosomes through an irregular meiotic division has led to 15 in *Oe. lata*, a characteristic mutation which has occurred both in *Oe. Lamarckiana* and in certain races of *Oe. biennis*. The same chromosome number occurs in *semilata* and in a very different form from Sweden which I have called *incurvata*.[2] DeVries[3] has recently described still another form having 15 chromosomes. It was derived from *Oe. biennis semigigas* pollinated in part from *Oe. biennis*, and has flat leaves, whitish foliage, white veins, longer spikes, slender buds, small, erect flowers, thin cylindrical fruits and few seeds. Hence we may say that whenever a germ cell having 8 chromosomes fertilizes a normal germ cell a new form is produced, though what its characters will be depends upon various circumstances which need not be considered here. One of the most important of these factors is probably the particular combination of chromosomes received. It is perhaps not inappropriate to speak of all these mutants as belonging to the *lata* series, or the series with an extra chromosome.

Oe. mut. *gigas* is the prototype of another series of still more closely parallel mutations in which the chromosome series is doubled—28—the plant being a cell giant and not merely gigantic in its external dimensions. This mutation has not only occurred independently in two different strains of *Oe. Lamarckiana*, but a somewhat different giant having the same chromosome number occurred in Heribert-Nilsson's cultures of the Swedish *Lamarckiana*. Recently Bartlett[4] has found a tetraploid giant in his experiments with *Oe. stenomeres* which is an exact parallel to the previous cases in every particular.

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[2] Op. cit., p. 147.

[3] DeVries, Hugo. 1915. The coefficient of mutation in *Oenothera biennis* L. Bot. Gaz. 59: 169–196.

A third series of morphological mutants is the *semigigas* series, having 21 chromosomes. This type of mutation has now been found in several species, including *Oe. Lamarckiana*, *Oe. biennis*, and hybrids of *Oe. Lamarckiana* or its derivatives with such species as *Oe. cruciata*, *Oe. muricata* and *Oe. Millersi*. Triploidy is thus much more frequent in its occurrence than tetraploidy, though the latter condition was discovered first.

Derivative from the above three types of chromosome change are various others. Thus a mutant from Sweden which I have called *latescens*[5] probably has 16 chromosomes, and by crossing and otherwise several additional numbers have been obtained.

The truth is obvious, not only that parallel mutations occur independently in different species, but that the type of change which gives rise to the extra-chromosome series is entirely different from that which produces the tetraploid series. I wish to point out that the nature of these changes is probably limited, and in this sense determined, by the structure of the germ plasm. In one group of organisms the mutations are of one kind, in another group they are wholly different in nature. This must be because the germ plasm in each group has its own particular lines of cleavage. Tetraploidy is a phenomenon which has occurred in a great variety of organisms, because the nuclear structure of almost any organism allows of its occurrence. Chromosome duplication, as in the *lata* series, is apparently much less common, but it may be expected to occur wherever the pairing of chromosomes in meiosis is weak and therefore liable to irregularities. Mutations then, in a sense, indicate where lines of weakness exist in the germ plasm, and it is these lines of weakness which define the particular directions which the mutations will take in any genus. Some of these "cleavage lines" are structural, some chemical in nature.

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[4] Bartlett, H. H. 1915. The mutations of *Oenothera stenomeres*. Amer. Journ. Bot. 2: 100–109, figs. 4.

[5] Op. cit., p. 117.

Another important feature of mutations which has not hitherto been emphasized is the fact that each is the result of a cell change which is represented in every part of the organism. This change originally occurred in the nucleus of a single cell, and the mitotic mechanism is responsible for handing it down to every part of the organism. The cells of *Oe. lata* constantly have 15 chromosomes, in whatever part of the plant they have been examined. Similarly in *Oe. gigas* even the most specialized tissues retain the double number of chromosomes transmitted to them,[6] though in the tapetal cells of all the forms secondary changes may take place, through fusion of nuclei and similar causes.

The conclusion follows that, with non-significant exceptions, every cell receives the number of chromosomes transmitted to it from the original fertilized egg. The blunt-pointed, deeply crinkled leaves, short stature, irregular branching, nearly sterile pollen, rounded buds and other features of *Oe. lata* are then an external expression of the fact that an extra chromosome is present in every cell. The real mutation was a cell change and is transmitted by mitosis as a cell change. Although we have at present practically no knowledge of the relation between cell structure and external form in organisms, yet we can at least affirm that the original change from a 14- to a particular 15-chromosome complex has resulted in the various external differences which we observe between *lata* and *Lamarckiana*. The organism is different because its every cell is different, and if in any part the extra chromosome should be dropped out into the cytoplasm through a slip in mitosis we should expect in that part a reversion to the foliage and other characters of *Lamarckiana*.

Viewed in this way, it is clear that we must consider the peculiarities of *lata* a result and not merely an accompaniment of the presence of the extra chromosome. We must, moreover, visualize the change as a cell change and the special features of *lata* as its external expression.

The same point of view applies probably to all other mutants. This makes comprehensible the fact that in many cases the mutants differ from the parent as strikingly in the early seedling stages as in the mature plant. The ontogeny of the new form does not witness a gradual drawing apart from its parent, except in so far as different organs may be unfolded in the later development. Already in the fertilized egg the difference is present, and makes itself felt as much in the first leaves as in the last. It is therefore important to remember that a mutant is such because not only its germ cells but every one of its somatic cells contains a certain peculiarity.

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[6] These and similar facts point to the conclusion that the chromosome divisions during ontogeny are not differential in nature, as Weismann supposed, but equational.

This is probably as true of animals as it is of plants, though an important difference is introduced here by the fact that in animals the germ cells are very early set apart from the somatic cells, while in higher plants this only happens with actual flower production.

If we compare the dimorphic condition of the cells in the males and females of certain insects with the difference between the cells of *Oe. lata* and *Lamarckiana,* it is clear that these differences correspond in certain features. The females of *Anasa tristis* have two members of a certain pair of chromosomes where the males have only one. Though the germ cells are set apart very early in the ontogeny in insects, yet it is probable that these chromosome differences occur not only in the young embryos, where they have been actually observed, but throughout the somatic tissues. A female animal, like a mutant, is somatically distinguished by having a different chromosome content in all its tissues, and not merely by the possession of female sex glands or secondary sexual characters. So far as I am aware, the fundamental significance of these facts in their bearing on heredity, embryology and variation, to say nothing of the structure of the cell, has not hitherto received attention, except in the case of cell giants. The fact that mutants differ from the parent in their every cell carries with it many important implications.

Giant mutations are now known in various organisms in the absence of tetraploidy. In such cases, although the cells are gigantic the chromosome number is unchanged. There is at present no clear indication of the fundamental nature of this type of cell gigantism. One of the best known instances is that of the giant variety White Queen Star, of *Primula sinensis*, described by Keeble.[7] This appeared in the normal variety and breeds true, but crossing experiments indicate that it may result from the presence of three independent factors. Various tetraploid giants of Primula have also been obtained.

No doubt there are many other types of chromosome change besides those I have mentioned. These for the most part await discovery or investigation, although the cytological literature abounds with cases which are probably of this nature. One such instance, recently described, may be referred to here. In one of the grasshoppers, *Tettigidea parvipennis*, Robertson[8] found that certain individuals possess an abnormally long chromosome mated with a short one, while in other individuals the corresponding pair are of equal length. The explanation appears to be that a portion from the end of one chromosome became attached endwise to its mate. A study of the variability of these grasshoppers should be made, to discover whether any corresponding somatic change can be detected in those that have the long chromosome. It is suggested that negative mutations might be due to the loss of a portion from the end of a chromosome in this way.

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[7] Keeble, F. 1912. Gigantism in *Primula sinensis*. Journ. Genetics **2**: 163–188, pl. 11.

In the remarkable phenomena of mutation in Drosophila studied by Morgan and his pupils, the new forms appear to result from changes in the nature of certain portions of particular chromosomes. The new characters are grouped in four series according to their hereditary behavior, corresponding to the four pairs of chromosomes, and in giving rise to each mutation a particular part of one chromosome may be assumed to have undergone a change.[9] This change is probably chemical in nature, at least in the series giving rise to the color varieties. The "crossing-over" phenomena, which have been studied in such detail by Morgan and his collaborators, are accounted for on the theory of the chiasma type of chromosome reduction, in which the chromosome pairs become looped around each other and so exchange segments of their substance. This process has not, I believe, been observed actually to take place in Drosophila.

In preparations of Drosophila chromosomes exhibited by Mr. Bridges, the somatic chromosomes appear to be remarkably closely paired and twisted about each other during the prophases of mitosis.

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[8] Robertson, W. R. B. 1915. Chromosome studies III. Inequalities and deficiencies in homologous chromosomes: their bearing upon synapsis and the loss of unit characters. Journ. Morph. **26**: 109–141, pls. 3.

[9] The recent observations of Chambers (Some physical properties of the cell nucleus, Science, n. ser., **40**: 824–827. 1914) on the living spermatocytes of a grasshopper lend direct support to the view that the chromosomes are composed of discrete and more or less independent particles. He found that stimulation of the cell induces chromosome formation in the resting nucleus, and that the chromosomes are formed by the aggregation of definite granules in bunches about a hyaline core.

The suggestion therefore occurs to one that it may be in the premeiotic rather than the meiotic divisions of the germ cells that the "crossing-over" of material from one chromosome to its mate occurs. Personally I have never seen such an intimate relation between the members of chromosome pairs as is to be observed in the somatic cells of Drosophila. The absence of crossing-over in the male is, however, a difficulty with any hypothesis yet proposed.

Returning now to the subject of Oenothera, there is at least one mutation which is fundamentally chemical in nature. In the origin of *Oe. rubricalyx* we see exhibited the type of change which must occur whenever a new monohybrid Mendelian character appears. In all such cases it is only necessary to assume that one chromosome, or a portion of one, underwent a change in its chemical nature. The meiotic mechanism performs the function of distributing this chromosome and its descendants so that a Mendelian 3 : 1 ratio in the offspring will result.[10]

There have been many suggestions as to why the new character is dominant in one case and recessive in another. Cases like that of *Oe. rubricalyx*, in which the new character is dominant, are rare. It is possible that dominance may occur whenever the change results in the increased production of a substance, and recessiveness whenever there is loss of or reduction in the capacity for producing such a substance in the cell. This would apply to both dominant and recessive whites. Thus in the case of dominant White Leghorn fowls a substance is present in quantity which inhibits color production, while in recessive white flowers it is clear that the capacity for producing color has in one way or another, been almost completely lost or suppressed.[11] One may suppose that this color-inhibiting substance is one of the substances produced by the cells in all Leghorn fowls, but that in the White Leghorn it has been very largely increased through a chemical modification on the part of a chromosome.

In the present state of our knowledge it is impossible to determine the precise chemical nature of the change which produced *Oe. rubricalyx* from *Oe. rubrinervis*. The difference consists in an enormously increased capacity for anthocyanin formation in every cell of the organism, but especially in the buds. When the chemistry of the nucleo-proteins is better known, it may be possible to determine what chemical change one of them would have to undergo in order to increase the amount of anthocyanin produced by interaction with the cytoplasm of the cell, but these matters are as yet too complex for analysis, though much is being learned concerning the chemistry of anthocyanin and the physiology of its production in the cell.

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[10] For an explanation of later 15:1 ratios, see Gates, 1915, On successive duplicate mutations. Biol. Bull. **29**: 204–220.

[11] Of course this suppression may occur in various ways. Thus it has been suggested (Robertson, J. B. 1914. Bloodstock Breeders' Rev. Nos. 1, pp. 16–31; 2, pp. 91–107; Reviewed in Exp. Sta. Record **32**: 361. 1915) that in the gray horse,

Like the morphological mutations to which reference has already been made, there can be little doubt that *Oe. rubricalyx* is also a cell mutation, the nuclei in all parts of the organism containing a descendent of the original changed chromosome. Parallels to this mutation are found in such plants as the copper beech and the red sunflower, which belong to widely separated groups.

In conclusion, our complete lack of knowledge of the relation between internal cell structure and external form in organisms may be pointed out. Except in the relatively simple case of gigantism through tetraploidy it is quite unknown how a change in the nucleus of the cell-unit results in the external modification of characters. Why are the buds of *Oe. rubricalyx* more conspicuously red than any other part, and why are the leaves of *Oe. lata* blunt, the buds rounded, the pollen sterile, etc.? Before an answer to such questions can be attempted, something must be learned of the way in which the metabolism of the cell—a complex series of chemical activities—expresses itself in the form of structure involving relationship between differentiated cells and tissues.

which is a recessive, the grayness results from a structural modification in the canals which connect the pigment-producing cells with the hair follicles, rendering them too narrow for the passage of the pigment granules.

THE RELATION BETWEEN VEGETATIVE VIGOR AND REPRODUCTION IN SOME SAPROLEGNIACEAE[1]

Adrian J. Pieters

Historical Introduction

Advances in science have been made by workers arriving at new points of view, as witness the work of Mendel, Darwin, deVries and others. So also the writings of Klebs during the last twenty-five years have served to open up a field, the cultivation of which is already exercising a profound influence on biological thought. Klebs was not the first to suggest that the forms, to the development of which a plant might at any one time devote its energies, were conditioned by external factors acting upon the plant; but it will be neither necessary not desirable to review the older literature. This has been adequately done by Klebs in his various papers. (See especially '00, '03, '04.)

The idea underlying all of Klebs's work is that every species has a sum total of potentialities constituting the specific nature of that species, but that the chemical and physical conditions prevailing at any one time within the organism determine which of the particular forms that the species is capable of producing shall appear, and that these inner conditions may be controlled, to an extent at least, by regulating the external conditions. In other words the changes in form which we witness in the course of the development of an individual are the results of chemical and physical interactions.

Since 1890, when Klebs ('90) showed that the formation and discharge of zoospores in Hydrodictyon could be encouraged by providing certain conditions, he has established the truth of this principle in the case of other algae ('96), of fungi ('98, '99, '00), and also for flowering plants ('03, '04). Other workers have built upon the foundations laid by Klebs and the present writer believes that the proposition that the succession of forms in the development of any plant is conditioned by environment, needs no further support than that furnished by the work of Klebs, Vöchting ('00), Kauffman ('08), Freund ('08), Obel ('10), and Raciborski ('96).

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[1] Contribution 150 from the Botanical Department of the University of Michigan. This work was begun at Heidelberg, Germany, under the direction of Prof. Dr. Georg Klebs, and was continued at the University of Michigan under the direction of Dr. C. H. Kauffman. To both of these inspiring instructors the writer wishes to return his thanks and to them he desires to express his appreciation of the mental stimulus he owes to their discussions and suggestions.

It has seemed worth while, however, to answer a question raised in the writer's mind by a study of Klebs's paper on *Saprolegnia mixta*. In this paper Klebs describes the production of sporangia and oogonia on a mycelium transferred from one solution to another, and states that such a mycelium, to react properly, must have been "well nourished." He does not define more exactly what he means by this term. Obel ('10) also emphasizes the need of a vigorous mycelium and uses a solution of peptone, sucrose and salts instead of the pea extract favored by Klebs. Obel agrees with Freund ('08), who states that, in algae, the conditions favoring the production of reproductive bodies depend upon the preceding conditions of growth and Obel states that this conclusion is confirmed by an examination of the Saprolegniaceae ('10, p. 427). Freund does not, however, give the results of any experiments to show the importance of the vigor of growth in the algae, and apparently makes the statement as a general one, meaning that the alga must be in good health and not in poor condition.

Neither Obel, Klebs nor Kauffman gives any record of experiments on this point. In his paper on *S. mixta* ('99) Klebs repeatedly refers to the necessity for a well nourished mycelium and states (p. 585) that during vegetative growth a part of the nutritive substance is stored, to be used later when reproduction shall have commenced. Further on the same page the statement is made that the production of oogonia requires a nutritive plasma (Nährplasma) of a somewhat different composition from that needed for the production of sporangia. Klebs fully recognized that the chemical nature of the solution to which a mycelium is transferred, is important. He found that haemoglobin and leucin were the substances most favorable to the production of oogonia and that the addition of certain phosphate salts increased both the number of oogonia and of antheridia. Kauffman ('08), in his work with *S. hypogyna* and with two cultures of *S. mixta*, did not find any marked increase in the number of oogonia due to the addition of salts, and the number of antheridia in the case of *S. mixta* was as marked in the solutions containing magnesium sulphate, as in those containing phosphates (l. c., p. 368). However, neither studied the effect of various foods consumed by the mycelium before transfer.

The Problem

The question was raised therefore, "What is a 'well nourished' mycelium?" and naturally the question of what constitutes a good nutritive solution followed. This work was commenced at Heidelberg, under the direction of Prof. Dr. Georg Klebs, who agreed with the writer that a culture medium, in which the fungus can attain the greatest dry weight in a given time under favorable conditions, is generally to be considered the best nutritive medium. The question which the writer set himself to answer therefore, is the following: Will a nutritive medium in which a species of water mold produces the greatest amount of dry matter in a given time also give to that mycelium when transferred to a suitable medium, the power to form more reproductive organs, than are produced by a mycelium grown in a poorer medium, measured by dry weight of mycelium produced? Or, in other words, is there a direct and necessary relation between vegetative growth and reproductive capacity?

Generally in cultures of other fungi the reproductive bodies, when formed under these conditions, are produced while the mycelium remains in or on the culture medium in which its vegetative growth has taken place; not so with the Saprolegnias. If satisfactory results in the production of sporangia or of oogonia are to be obtained, these plants must be transferred from the solution in which they have been grown, to another and weaker solution. When the plant is transferred to the second solution, it finds itself in a new environment. This may differ from the old merely in concentration and in the absence of the poisonous products of metabolism, which have accumulated in the old solution, or it may differ from the latter in chemical composition. As Klebs has pointed out ('99), whatever the chemical nature of the second solution may be, it must, if oogonia are to be formed, be of such character and concentration, that a slight growth can take place. If the solution is too poor in food, sporangia will appear and perhaps exhaust the mycelium, while if the food is too good vegetative growth will prevent reproduction.

If the protoplasm produced by the consumption of all kinds of foods is alike we may expect that the nature of the changes observed after transfer will depend wholly on the new environment; but if the food used during vegetative growth can affect the character of the protoplasm, as well as the weight of the product, such effects should become evident when mycelia, grown in different culture media, are transferred to solutions of identical composition and concentration. The new and uniform environment acting upon the protoplasmic product of varying environments may be expected to bring out differences if any exist. Data will be presented in this paper to show that, in some cases at least, such an effect, not to be measured in terms of dry weight produced, has resulted from the use of special culture media and that there is no direct and necessary relation between weight of mycelium and the production of oogonia.

It was realized at the outset that there were two aspects of the problem—the quantitative and the qualitative. Either a nutritive substance of one kind might affect the organism because of its greater abundance, or substances of various kinds might affect especially the reproductive capacity or the vegetative growth.

Solutions were therefore prepared with these two aspects in view, different concentrations of peptone being used on the one hand and various carbohydrates and salts on the other. The number of oogonia produced on the mycelia after transfer, together with the weight of mycelium in the duplicate lots left in the nutrient solutions, make up the evidence on which the conclusions reached are based; this evidence can best be presented in a series of tables and as tables are rather uninteresting reading, the writer has pointed out briefly after each table the conclusions that seem warranted by the evidence, leaving a general discussion of these conclusions to be given at the end of the paper. The chief contributions which it is believed are made by the present work to our knowledge of the physiology of these forms are the recognition of a minimum concentration of food necessary for a full development of the plant, and the fact that above this minimum an increased concentration during vegetative growth does not necessarily increase the reproductive capacity of the fungus. It developed further, as will be shown in the following pages, that certain carbohydrates are readily used while others can not be utilized, and that of those that are used some are of more value to the plant for the formation of reproductive organs than others.

MATERIAL

The material used for this study consisted of four species of Saprolegniaceae, selected from among a larger number isolated and studied.

Two of these belonged to the genus Saprolegnia. *S. ferax* (Gruith) Thuret was collected at Heidelberg in the fall of 1911; *S. monoica* Pringsh. was secured from alga material out of pools near Ann Arbor. *Achlya racemosa* Hildebr. was collected at Heidelberg and was unfortunately lost in a fire which destroyed a large part of the botanical laboratories at Ann Arbor in the spring of 1913, at which time also a large number of notes and other records were lost. The records of this species are therefore incomplete. *Achlya prolifera* De Bary was obtained from alga material out of pools near Ann Arbor. Physiological studies on several other species, together with descriptions of some new species and remarks in regard to certain points of relationships, will be discussed in other papers.

METHODS

For isolating the forms Kauffman's ('08) method of single spore cultures was strictly followed. While the writer is not disposed to say that men like Maruzio ('94) and Obel (l. c.) did not work with pure cultures it seems more than likely that the results claimed to have been gotten by Lechmere ('11) may be due, in part at least, to mixed cultures. Moreover, a writer with so little appreciation of the worthlessness of sporangia and gemmae as specific characters,[2] can hardly be taken seriously when he states that the sporangia characteristic of six different genera were found in one supposedly pure culture. While Lechmere says that he used Kauffman's method he has apparently quite overlooked the fact that the essential part of this method is the isolation of one spore; it does not appear that he attempted to do this in his work.

All the cultures of each species made in connection with the present problem were descended from an original single spore and if there was the slightest reason to doubt the purity of a culture, a single spore culture was again made and carried through on a fly, so that the specific characters might be compared with the original. All the species that were not being used for experimental work were kept alive on flies or in pea extract. In either case cultures can be set aside for two or three months without any danger of loss.

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[2] See l. c., p. 176, where Lechmere concluded that two species of Saprolegnia were identical because of the similarity of habit, gemmae, and shape and peculiarities of the sporangia, although the one produced oogonia readily and the other did not produce them at all.

For the experiments cultures were made in Erlenmeyer flasks of Jena or of resistance glass except in the few cases that will be noted, the flasks containing 100 or 200 cc. of culture fluid. This medium was inoculated by placing in it small bits of pea agar in which the fungus was actively growing. Pea agar was used instead of the beef gelatin used by Kauffman as it was found more convenient, both to make and to handle. It is easily made by putting about 100 peas in a liter of water together with sufficient agar to make the strength desired. This is heated in an autoclave for an hour at about 15 to 20 pounds pressure and, after filtering, is ready for use. It has been found a good culture medium, and has a further advantage over gelatin in not becoming soft in warm weather and in not being liquefied by the action of the fungus. When a set of cultures was to be inoculated the bits of agar were cut as nearly of the same size as possible, about 1 square millimeter in size. After having grown for the necessary length of time the entire mass of mycelium was placed in a dish of sterile distilled water and allowed to stand for half or three quarters of an hour. It was then cut into pieces of as nearly the same size as possible and usually of about one square centimeter in area. These pieces were transferred to fresh sterile distilled water and were sometimes washed a third time before being placed in the solutions in which sporangia or oogonia were to be made. For the latter solutions glass capsules containing 25 cc. of solution were used. All of these cultures were in duplicate, and sometimes in quadruplicate.

Condition of the mycelium.—For studying the effect of food previously consumed, on a mycelium after transfer to haemoglobin, or to some other suitable medium for the production of oogonia, or to water for the production of sporangia, it is important that the mycelium be actively growing, and that, in all the experiments, it shall be as nearly as possible in the same physiological condition. If a flask containing 100 cc. of pea extract be inoculated, the resulting growth will reach the surface after four or more days, depending upon the species. When the surface of the liquid is reached, a mat is formed, which, after a few days, may become quite thick and firm. At this stage the mycelium is in a very different condition from that in which it is just before reaching the surface. In the latter case the hyphae are all actively growing, the tips being, of course, the most active; when a mat has formed, and especially if the mat has been allowed to become thick, the lower parts of the mycelium are dead or dying while the parts in the mat have taken on something of a resting condition. If such a mat is cut up and the pieces placed in another solution, new growth must take place in that solution before sporangia or oogonia can be formed, but if the mycelium is used earlier the hyphae not only grow but respond at once to the stimulus of the new environment by the production of appropriate organs. It was found that the most advantageous stage at which to use a mycelium was just as the mat started to form, for at that stage masses of actively growing hyphae, all in uniform condition, can be readily secured.

Temperature.—Temperature is a factor of the utmost importance. Klebs states that a temperature of about 18 degrees Centigrade is the optimum for *S. mixta*, but this is certainly not true for *S. ferax*. It has not been possible to determine the optimum for each of the various species studied, but enough was learned to warrant the statement that the temperature requirements of the different species differ. In *Achlya racemosa* a high temperature favors the production of oogonia and inhibits the formation of sporangia, while the temperature at which oogonia are produced with certainty by *S. ferax* is in the neighborhood of 15 degrees. *S. monoica* seems to do about as well at 18 degrees as at 15; but at 20 degrees or over there is a distinct decrease in the number of oogonia. *Achlya prolifera* seems to prefer a temperature of 18 to 20 degrees; at lower temperatures as well as at higher, 22 to 24 degrees, the production of oogonia is uncertain. Unfortunately it was not possible to determine the optimum temperatures from lack of apparatus by means of which constant low temperatures could be maintained. For the purposes of the present work, however, it was sufficient to maintain the temperatures near the optimum for each species. It is interesting in this connection to note that Peterson ('10) in his study of the Danish freshwater Phycomycetes states that *A. racemosa* produces oogonia with difficulty, and only at low temperatures. In the writer's experience *A. racemosa* produces oogonia very readily and particularly so at relatively high temperatures.

Method of expressing the number of oogonia.—Klebs, in comparing the value of different substances for the production of oogonia expressed the number of oogonia by the Roman figures I, II, and III, the relative number of oogonia present being in that order; Kauffman and Obel merely stated that few or many oogonia were present, while Horn ('04) followed the precedent set by Klebs. Such a method does very well when one wants only to say that a food is poor or very good, but obviously if the number of oogonia formed is to be used as a measure of the value of a food, a more exact method of determining that number is necessary. In the present work the method of Klebs was used to some extent, especially in the earlier part of the work, but it soon became evident that this was not sufficiently accurate. When the value of various foods for vegetative growth is to be determined, the mycelium is dried and weighed at the conclusion of the experiment, but it is not practicable to weigh the oogonia produced; these were therefore counted. For this purpose the mycelium was removed from the dish in which it was growing and laid on the inside of a Petri dish cover. Here it was carefully spread out by means of a jet of water from a pipette till it lay as a circular flat mass, the older, and thicker, portion at one end or near the middle and the younger portions forming a fringe around this.

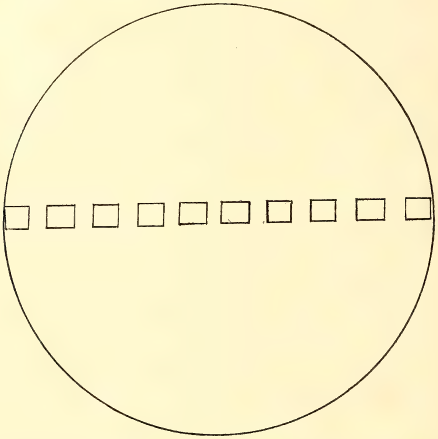

FIG. 1. Diagram showing the arrangement of the spaces in which the oogonia were counted.

The bottom of the Petri dish was then inverted and laid on the mycelium, so that the whole lay flat and firm between the two halves of the Petri dish. A square ruled micrometer had been fitted to the eyepiece of a Leitz compound erecting body and the number of oogonia in a square, having an area of 25 square millimeters, was determined under a Leitz number two objective. The oogonia are, of course, not uniformly distributed over the mycelium, and in order to find the average number, the oogonia in ten squares were counted, beginning at one edge and following a line across the mass. As a rule the oogonia are more numerous a little back from the edge than in the middle, but this is not always the case.

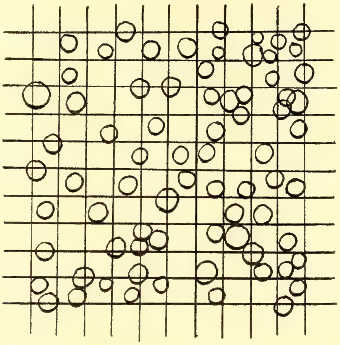

FIG. 2. Diagram showing the arrangement of the oogonia in one of the spaces. Magnified about 100 times.

The diagrams, text figures 1 and 2, show the method of counting and Table I gives some counts showing the distribution of the oogonia on the mycelium. This matter will be referred to again.

TABLE I

Illustrating Method of Counting Oogonia. Each Number Represents the Oogonia in One Square of 25 *Square Millimeters. Oogonia out of Haemoglobin* 0.075 *Percent or Haemoglobin* 0.05 *Percent*

From Set III

Peptone and levulose	40,	85,	63,	52,	81,	87,	144,	115,	82,	37—	Ave. 78.6
Cross count	14,	41,	26,	145,	92,	76,	76,	86,	66,	49—	" 67.1
Duplicate	44,	80,	88,	72,	79,	85,	70,	76,	76,	85—	" 75.8
From pea	3,	10,	11,	12,	78,	76,	60,	61,	72,	37—	" 42.1
Cross count	4,	8,	13,	15,	22,	79,	122,	97,	37,	27—	" 42.4
Duplicate	6,	65,	89,	80,	57,	75,	66,	23,	13,	19—	" 49.3

From Set VIII

Peptone and levulose.........42, 84, 97, 87, 120, 72, 135, 150, 209, 191—Ave. 118.7

The advantage of this method lies in the fact that the mycelium studied is practically all in one plane of focus, only a slight turn of the focusing screw being required to enable the observer to count every oogonium. That such counting of oogonia was extremely tedious and time consuming is quite obvious; consequently the quicker method of Klebs was also used. In the following tables wherever the Roman numerals are used they should be interpreted as follows:

I. A fair number, about a dozen in the field of a Leitz number two objective.
II. Many, at least four or five times as many as I.
III. Very many, at least twice as many as II.

To express intermediate gradations, the following have been used: 0–I, a few in each field; I–II and II–III to indicate numbers not properly expressible by either figure alone.

Transferring the mycelium.—In each set of experiments the mycelium was removed from some flasks five to six days after inoculation, washed, cut up and transferred to haemoglobin for the production of oogonia, or to some other solution as will be indicated in the proper place. The mycelium in the duplicate flasks was allowed to grow for some time longer and was then washed, dried at 100 degrees and weighed. The length of time the fungus was allowed to grow before being weighed was, of course, precisely the same for every lot of a series; with the exception of series I this time was thirty days. The capsules containing the haemoglobin or other solution were always in duplicate, and sometimes in quadruplicate, and were kept at a temperature as nearly uniform as possible. This varied roughly between 13 and 20 degrees, the extremes being only occasionally reached; usually the temperature was between 15 and 18 degrees. The first experiments were made at Heidelberg and the mycelium was not weighed, nor were the oogonia counted. The later experiments were made at Ann Arbor.

Klebs showed that the most important condition for the production of oogonia by *S. mixta* was a gradual decrease in the food supply and that for the formation of sporangia a sudden, or at least a rather rapid decrease in the concentration of the food, was necessary. To secure oogonia, then, the mycelium must be placed in such an environment that, while no rapid growth takes place, the formation of sporangia shall be almost or entirely prevented. It is evident that a mycelium, exhausted by the formation of sporangia, cannot produce the number of oogonia that it could have produced under other conditions. Haemoglobin, in concentrations to suit, offers such a medium, but unfortunately the commercial article is not of uniform purity, always leaving more or less of an insoluble precipitate. Klebs ('99, p. 520) states that in 0.05 percent haemoglobin only vegetative growth took place and that even in 0.01 percent sporangia were but sparingly produced. The forms of *S. mixta* collected by the writer formed sporangia readily in 0.05 percent haemoglobin and some even in 0.075 percent, and this was also true of *S. ferax* and of *S. monoica.* While Klebs's form may have developed sporangia less readily than those which the writer used, the difference in the results is doubtless due largely to the relative purity of the haemoglobin. As a consequence concentrations thought to be the same are not always so, and, owing to the delicate balance between growth and sporangia formation existing in a weak solution, a slight difference may result, either in the formation of many sporangia, and in the consequent weakening of the mycelium, or in an undue amount of growth with the attendant formation of the poisonous products of metabolism. In either case the formation of oogonia will be interfered with.

Purified water.—The earliest tests made in Heidelberg brought out the fact that distilled water from the chemical laboratory had a harmful effect on the mycelium. Horn ('04) had already observed this and had attributed it to the presence of copper, or of some other metal in the water. He found that when common distilled water was re-distilled out of hard Jena glass, it lost its poisonous properties. This was not found to be so in the present case. Distilled water was twice re-distilled out of hard Jena glass, but, although the latter was better than the distilled water as it was received from the chemical laboratory, it was by no means as good as freshly collected rain water. The action of the distilled water was not uniform, sometimes wholly preventing the formation of sporangia, while at other times some sporangia were formed but did not discharge normally. This was also found to be true sometimes of the rain water, which, though often satisfactory, was not infrequently quite as toxic as the distilled water. Much of the work first done had to be discarded because of this unreliable action of the water. The use of carbon black as described by

Livingston ('05) was then tried and it was found that this gave uniform and satisfactory results. Out of the many records available on this point only one table will be given to show the effect of the water on the formation of sporangia. Tap water was also found to be quite as unsatisfactory as distilled water.

TABLE II

Achlya racemosa, Grown Three Days in Culture Solution N[3]; Transferred to Water for the Formation of Sporangia

1	2 Sporangia in 12 Hrs.	3 In 24 Hrs.	4 48 Hrs.
Distilled water from chemical laboratory.....	0, Mycelium dying.	Dead.	
Distilled water shaken up with carbon black......	I + discharged.	III	Exhausted.
Distilled water re-distilled and filtered through carbon black............	I + discharged.	III	Exhausted.
Double distilled rain water.	Few formed, none discharged.	Few discharged.	Some gemmae.
Double distilled rain water filtered through carbon black.................	II + discharged.	III	Exhausted.

Similar results were secured with other species. All species are not equally sensitive to the water, however, but all gave more uniform results when water purified with carbon black was used. In all the following experiments, therefore, whenever the ability of a mycelium to produce sporangia was to be tested purified water was used.

To determine whether the use of this carbon black would introduce an unknown food element into the water some carbon black was carefully washed by shaking it up with a small quantity of water, which was then filtered off. Mycelium was transferred to this water but in no case did any growth result, though sporangia were at once formed showing that the water contained no food.

Culture media.—With the exception of pea extract, only synthetic media were used. Many of the various natural media, such as ants' eggs, larvae, and so forth, might have been used and might have given interesting results. It was desired, however, in the first place to use, so far as possible, only media of known composition; and in the second place to weigh the mycelia. Any insoluble substances would introduce an element of error by adhering to the fungus hyphae. When pea extract was used this was prepared by sterilizing about six peas with a hundred cubic centimeters of distilled water. If carefully sterilized, the peas did not break up. Any flasks in which the peas had broken up were discarded for these experiments, as the loose starch grains would surely adhere to the fungus and increase the weight. Peptone was used as a source of both nitrogen and carbon and in a few cases haemoglobin was used. Asparagin and leucin were tried but the growth was so poor that these substances were not considered satisfactory; Klebs ('99, p. 520–523) also found that growth took place in lower concentrations of peptone than of leucin. The latter is very expensive to use for so large a number of experiments as had been planned, and is moreover of a rather uncertain quality. In the course of the work there was occasion to try the leucin from three prominent German makers and no two were found to give like results. Synthetic leucin was once used, but proved to be so toxic that little growth took place in it. Carbohydrates were added as an additional source of carbon. At first sucrose alone was used, but later maltose, levulose and dextrose were added. For salts, potassium nitrate, magnesium sulphate, and potassium phosphate, either mono- or di-basic were added. Wherever in the tables the term "salts" is used, the above salts, in concentration to total 1/100 or 1/200 molecular, are meant. In the earliest experiments these salts were used in a strength of 0.1 percent or 0.05 percent but later molecular weights only were used. In some cases each salt was used separately.

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[3] This solution contained 0.1 percent peptone, 1 percent sucrose and 0.1 percent salts.

EXPERIMENTAL

Experiments with Achlya racemosa

This species offers advantageous material because it will produce in water oogonia with oospores and antheridia. It is not necessary to transfer to another food solution; it is only necessary to prevent the too rapid formation of sporangia. This can be done by placing the mycelium in a temperature of 24 degrees, at which temperature few sporangia are formed, while the formation of oogonia is not interfered with. The mycelia for the following experiments were grown for seven days in 50 cc. of the following solutions.

(1) Peptone 0.1 percent, sucrose 1 percent, salts 0.1 percent; (2) peptone 2 percent; (3) peptone 1 percent; (4) peptone 0.5 percent;

(5) peptone 0.1 percent; (6) peptone 0.05 percent; (7) peptone 0.01 percent; (8) peptone 0.1 percent, salts 0.1 percent; (9) peptone 0.1 percent, salts 0.05 percent; (10) peptone 0.1 percent, sucrose 1 percent; (11) peptone 0.1 percent, sucrose 0.5 percent.

At the end of seven days a mat had been formed on the surface of the solutions, numbered 1, 8 and 9 while the quantity of mycelium was in the following order, numbers 1, 8, 9, 2, 3, 4, 10, 11, 5, 6, and 7.

As before stated, no weighings were made for this species, but careful notes were taken of the apparent mass and condition. From these it appears that the complete solution, such as was suggested by Obel, solution number 1, produced the best growth; that a mat was formed only in those solutions containing salts and that those solutions were much better for growth than those containing the same amount of peptone and sugar, but no salts. There was very little difference between the mass of mycelium produced by solutions of 0.5 percent or 1 percent peptone or by peptone and sucrose, but the mass of mycelium out of 2 percent peptone was many times greater

Table III

Achlya racemosa. Mycelia Grown Seven Days in Media Given in Column 2. Transferred to Water at 18°

1	2	3	4	5	6	7
		Sporangia, 1 Day.	Sporangia, 2 Days.	Oogonia, 1 Day.	Oogonia, 2 Days.	Oogonia, 5 Days.
1	Peptone 0.1%, sucrose 1%, salts 0.1%	II–III	II[4]	0–I	I	I[5]
2	Peptone 2%	II	II	0–I	0–I	0–I
3	Peptone 1%	II–III	I	0–I	I	II
4	Peptone 0.5%	II	III	I	I	II
5	Peptone 0.1%	0–I	0	I–II	II	III
6	Peptone 0.05%	0	0	II	II–III	III
7	Peptone 0.01%	Very few	0	II	III	III oogonia mostly empty
8	Peptone 0.1% + salts 0.1%	II–III	0–I	I	III	III
9	Peptone 0.1% + salts 0.05%	II+	I	0	I	I
10	Peptone 0.1% + sucrose 1%	0	0	I	II	III
11	Peptone 0.1% + sucrose 0.5%	0	0	II	II	III oogonia mostly empty

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[4] The number of sporangia given for the second day is in addition to those first recorded.

[5] The number of oogonia recorded for each day represents the number then present and not a number in addition to those previously recorded.

than that out of 0.01 percent peptone, as was to be expected. After being washed the mycelia from these solutions were transferred to water at the laboratory temperature, about 18° C. Sporangia were readily produced and Table III shows the relative number of sporangia and oogonia from mycelia grown in various culture media.

With the exception of the mycelium out of 2 percent peptone, there is roughly an agreement between the mass of mycelium and the number of sporangia formed, and it is clear that a poorly nourished mycelium will not make many sporangia. The addition of salts to the peptone not only increased the vegetative growth but also the number of sporangia, a result not secured with sugar. So far as could be observed the mycelium from the 0.5 percent peptone developed as many sporangia as did that from 1 percent or from 2 percent, while that from 0.1 percent showed a falling off. This would seem therefore to be the critical concentration, below which a still more decided falling off in the number of sporangia is to be noted. The same mycelium developed both sporangia and oogonia in water, and the

TABLE IV

Achlya racemosa. Mycelia Grown Seven Days in Media Under Column 2. Transferred to Water at 24°

1	2	3	4	5	6	7	8
	Media.	Sporangia, 1 Day.	Sporangia, 2 Days.	Oogonia, 1 Day.	Oogonia, 2 Days.	Oogonia, 5 Days.	Remarks.
1	Peptone 0.1%, sucrose 1%, salts 0.1%	Few	0	II	III	III	Many without oospores.
2	Peptone 2%	I	I	I	I	II	Oogonia larger than in No. 1 and well filled.
3	Peptone 1%	0–I	0–I	II	II	III	Oogonia large, mostly with one large oospore.
4	Peptone 0.5%	I–II	0	II–III	III	III	
5	Peptone 0.1%	Very few	0	II–III	III	III	As in number 3.
8	Peptone 0.1%, salts 0.1%	0	Rare	II–III	III	III	Some empty oogonia.
9	Peptone 0.1%, salts 0.05%	0	0	II–III	III	III	About 20% empty.
10	Peptone 0.1%, sucrose 1%	0–I	0	III	III	III	About 30% empty.
11	Peptone 0.1%, sucrose 0.05%	0	0	III	III	III	About 50% empty.

table shows that there was no agreement between mass of mycelium and number of oogonia, which fact may, however, have been due to the exhaustion of the mycelium by reason of the sporangia produced.

By keeping the mycelia after transfer to water at a temperature of 24°–25° C. the production of sporangia could be almost wholly prevented. This was done and the result is given in Table IV. The cultures used in this case were duplicates of those used for the previous experiment.

It will be noted that at this temperature the production of sporangia was almost inhibited. The number of oogonia present at the end of five days was in every case great; but it was also noted that the oogonia on mycelium from 1 percent and from 0.1 percent peptone were large, though containing but one oospore, while in all other cases, except the mycelium from 2 percent peptone there were many empty oogonia, sometimes as many as 50 percent. In this series the largest number of oogonia, containing oospores, was produced by the mycelium having the smallest mass.

From the same culture series another set, now seventeen days old, was taken and placed in water. After five days the number of oogonia found was as shown in Table V.

TABLE V

Achlya racemosa. Mycelia Grown Seventeen Days in Media Under Column 2. Transferred to Water at 18°

1	2 Media	3 Oogonia in 5 Days
3	Peptone 1%	III, oospores 2–3 in an oogonium.
4	Peptone 0.5%	III, large, 2–4 oospores.
5	Peptone 0.1%	II–III large oospores.
6	Peptone 0.05%	II, smaller than in 0.1 and few oospores.
7	Peptone 0.01%	I–II, few with oospores.
8	Peptone 0.1%, salts 0.1%	II–III, large and good. There have also been many sporangia.
9	Peptone 0.1%, salts 0.05%	0–I, there have been many sporangia.
10	Peptone 0.1%, sucrose 1%	II, small, but with oospores.
11	Peptone 0.1%, sucrose 0.5%	II, mostly with oospores.

The table shows that, while above a certain concentration there is no relation between mass of mycelium and the number of oogonia produced, there is a concentration below which the lack of food is manifested in the smaller number of oogonia formed. In the case of

Achlya racemosa, this limit lies between 0.1 percent and 0.05 percent peptone. Above this limit there was an increase in the growth, whether the concentration of the peptone only was increased, or whether salts or sugar were added, but there was no corresponding increase in the number of oogonia produced. Results similar to those given in the above tables were secured from other tests and we may conclude that for *Achlya racemosa* there is a concentration of food which, while it will enable the fungus to make some growth, will so reduce its vigor as to interfere markedly with the production of sporangia and of oogonia. Above this critical concentration the effect of the food will be marked by an increase in weight without a corresponding increase in the number of sporangia or of oogonia formed when the mycelium is transferred to water. For the production of the maximum number of sporangia this concentration lies above 0.1 percent peptone while for the production of oogonia it lies between this and 0.05 percent peptone.

Experiments with Saprolegnia ferax (Gruith) Thuret

This species was collected at Heidelberg in the fall of 1911. One spore was isolated, and all the cultures made during the past three years have been with material derived from this original culture. This species was first clearly separated from other members of the "ferax group" as *S. Thureti* by DeBary in 1881 ('81) when he called attention to the fact that antheridia were scarcely ever found. Later ('88) he distinguished *S. mixta* as having antheridia on about one half of the oogonia and as having a more slender mycelium than that of *S. ferax*. A careful study of the form collected as number 25 failed to disclose antheridia and a comparison of this form with another collected as number 17 led to the conclusion that number 25 should be identified as *S. ferax* while number 17 had the characters of *S. mixta*. Humphrey ('92) has discussed the nomenclature of these species and since his point seems well taken the name *S. ferax* (Gruith) Thuret is used here instead of *S. Thureti* as this species was called by DeBary. The fact that antheridia were found later has been recorded in a paper read before the Michigan Academy of Science at the meeting of April 1915, together with a discussion of the "ferax group" of the genus Saprolegnia (see Pieters, '15).

S. ferax furnishes favorable material, since the oogonia are quite large and are readily produced. Klebs had already shown that

S. mixta produced oogonia readily in 0.05 percent haemoglobin and Kauffman that *S. hypogyna* produced oogonia readily under similar conditions. Preliminary tests showed that *S. ferax* behaves like the above species and in all subsequent work haemoglobin, either alone or with salts and sugars, was used as the transfer solution when it was desired to test for oogonia. Unfortunately commercial haemoglobin is not of uniform purity and it sometimes happened that a solution made up to 0.05 percent was stronger or weaker than expected and thus either encouraged unusual growth or the formation of sporangia. Either event worked disadvantageously for the production of oogonia.

The different tests could not of course be carried on at the same time and hence were subject to variations in temperature. Klebs showed that a temperature of 18° C. was favorable for the production of oogonia in *S. mixta* but under the conditions prevailing in the laboratories of the University of Michigan such temperatures are almost impossible to maintain. The room temperature is generally 20° C. and above, temperatures at which oogonia are not readily produced. Some tests were therefore carried on in the basement of a private house while others were conducted in a small closet built in from a window in the botanical laboratory and opening to the outside by a swinging window. By doing the work in the winter and by using an electrically heated incubator, fairly satisfactory temperatures could be maintained but not exact enough to reach any conclusions as to the optimum temperatures for reproduction. The following record will, however, show the difference between the number of oogonia produced at lower and at higher temperatures. Mycelia

TABLE VI

Saprolegnia ferax. Mycelium Out of Media Under Column 1. *Transferred to Haemoglobin and to Haemoglobin and Salts at Different Temperatures. Average Number of Oogonia in* 25 *Square Millimeters*

1	2	3	4	5
Medium	20°–23° C. Haemoglobin 0.05%	14°–18° C. Haemoglobin 0.05%	20°–23° C. Haemoglobin 0.05%, Salts 0.1%	14°–18° C. Haemoglobin 0.05%, Salts 0.1%
Peptone 2%	13	70	—	198
Peptone 1%	19	67	15	142
Peptone 0.1% + Knop's Sol. 0.05%	15	176	5	157
Peptone 0.2% + sucrose 1%	5	51	4	163
Peptone 0.1% + sucrose 0.1%	21	59	15	155

from various solutions were transferred February 14, 1913 to 0.05 percent haemoglobin and to haemoglobin and salts, one set being kept in the laboratory where the temperature ranged from 20° to 23° C. and often higher while another set was kept in the basement of the writer's home at a temperature varying between 14° and 18° C.

Production of sporangia.—The optimum temperature conditions for the production of sporangia are quite different, a high temperature causing the sporangia to develop more rapidly than a low one. Klebs found this to be the case in *S. mixta* also. At a temperature of 28° C. *S. ferax* formed young sporangia in five and one-half hours and these were discharged in seven hours from the time the mycelium was put into water. A duplicate lot from the same culture, kept at laboratory temperature (about 20° C. at that time), formed young sporangia in seven hours and these were discharged in nine hours. The maximum temperature at which sporangia were formed in this species was 30°+ C. at which some swarming took place in six and three quarter hours but the spores left the sporangium slowly and were evidently feeble. In another test duplicate lots of mycelium were placed in water at 28° C., at laboratory temperature 18°–20° C., and in an ice box where the temperature was between 6° and 8° C. Table VII shows at the same time the effect of the higher temperature on the production of sporangia and also the relative number of sporangia formed in mycelia out of different foods.

Table VII

S. ferax. Mycelia Out of Media Under Column 1. *Transferred to Water at Different Temperatures for the Production of Sporangia*

1	2	3	4	5
	At 28°. 16 Hrs.	18°–20°. 16 Hrs.	6°–8°. 16 Hrs.	6°–8°. 22 Hrs.
Peptone 0.1%, sucrose 1%, salts 0.1%	III	III	II, none discharged	III, just discharging.
Peptone 2%	III	III	I, none discharged	I, none discharged.
Peptone 0.5%	II+	II+	I, none discharged	I, a few discharged.
Peptone 0.1%	II+	II+	I, none discharged	I–II, a few discharged.
Peptone 0.05%	I	I	0	0–I, a few discharged.
Peptone 0.01%	0–I	0	0	0, a few discharged.
Peptone 0.1% + sucrose 1%	III	II	0–I, none discharged	I–II, discharging.
Peptone 0.1% + salts 0.1%	III	III	0–I, none discharged	I, discharging.

The fact that no examination was made before sixteen hours explains why there is no apparent difference between the number of sporangia produced at 20° and at 28° C. It may also be noted how the higher temperature helps to conceal the difference in strength between the well- and the poorly-nourished mycelia. When many sporangia are formed at high temperatures it is really impossible for the observer to make a close comparison between them. At the low temperature, however, the difference in vigor is more clearly brought out. Here again, as noted before for *Achlya racemosa*, the difference between mycelia grown in 2 percent, 0.5 percent, and 0.1 percent peptone is not very marked though the first had food at a concentration twenty times greater than the last. But as soon as the concentration of the food falls below 0.1 percent the effect is quite marked; the critical concentration lies between 0.1 and 0.05 percent.

Production of oogonia.—It will be necessary before presenting the results of the experiments in which oogonia were counted to discuss more fully than was done earlier the reliability of the method of counting the oogonia. When a mycelium is spread out between two glass plates the oogonia are sometimes scattered quite uniformly over the surface of the plate, but more often the largest number of oogonia lie near but not just at the margin and again near the thickest part of the mass, which is the original piece put into the dish of haemoglobin. When the oogonia are uniformly scattered the count can be made along any line, but sometimes the observer must select a line that will lie across an average field or must count along a second line at right angles to the first. It may be asked whether such a count will accurately represent the number of oogonia on a given mycelium. The answer to this is that with few exceptions two counts of oogonia on lines at right angles to each other agree closely. It may also be asked whether such a count means anything, whether it is not rather wholly fortuitous and whether another lot under the same conditions might not show a very different number. It must be admitted that there is an element of uncertainty here which the writer would have been glad to eliminate. Living forms do not always behave with apparent uniformity; cases occurred in which the mycelium in one dish of a pair showed many oogonia while on the duplicate not one was to be found. Such instances however were exceptional. As a rule the number of oogonia was nearly the same in each dish of a pair as will be seen from the tables and it is believed that where duplicate pieces of mycelium placed under identical conditions give similar results, such results may be assumed to correctly represent the response of the plant to these conditions. In Tables VIII and IX a number of figures are given to show that counts at right angles to each other agree substantially and that the counts from duplicate dishes also agree pretty closely. While the duplicate dishes were not always counted, because of the great amount of labor involved in this work, they were always examined and when the number of oogonia was strikingly different from that in the dish counted this fact was noted. In all other cases the oogonia were either counted or the fact noted that the two dishes were in substantial agreement. No oogonia were counted that did not contain oospores. Table VIII has been compiled from the record of Series III transferred November 19, 1914. In no case is there a great difference between the counts at right angles to each other, and only in numbers 6, 7 and 12 is there any considerable difference between the number of oogonia in the two dishes of a set. Such a difference was often due to contamination of one dish by bacteria, but whatever the cause, whenever there was as great a variation as is shown by numbers 6, 7 and 12, the highest average was used in the final tables.

TABLE VIII

S. ferax. Mycelia Out of Media Given Under Column 2. Transferred to 0.075 *Percent Haemoglobin. All Sugars in the Culture Media* 1/50 *Molecular. Temperature* 16°–18° *C. All Figures Given to the Nearest Whole Number*

1	2	First Dish			Second Dish			9
		3	4	5	6	7	8	
	From Medium	First Count	Cross Count	Average	First Count	Cross Count	Average	Average of All
1	Pea	42	42	42	49	—	49	46
2	Peptone 0.1%	19	—	19	23	—	23	21
3	Peptone 0.2%	49	44	47	45	—	45	46
4	Peptone 0.5%	54	41	47	27	31	29	38
5	Peptone 1%	41	39	40	43	—	43	41
6	Peptone 0.1% + sucrose	25	34	29	11	15	13	21
7	Peptone 0.1% + maltose	65	65	65	25	28	27	46
8	Peptone 0.1% + dextrose	29	28	29	28	—	28	28
9	Peptone 0.1% + levulose	79	67	73	76	—	76	74
10	Peptone 0.2% + sucrose	19	26	22	36	32	34	28
11	Peptone 0.2% + maltose	46	46	46	40	—	40	43
12	Peptone 0.2% + dextrose	23	25	24	8	13	11	17
13	Peptone 0.2% + levulose	33	29	31	55	—	55	43
20	Haemoglobin 0.1%	23	22	23	21	—	21	22

In Table IX another record is given to show that there is substantial agreement between the number of oogonia found on mycelia from like origin and that mycelia grown in different media may vary in their capacity to produce oogonia. It is not urged that the figures obtained have the value of those commonly secured in a seed test, for example, for there are too many unknown and uncontrollable factors; but they are offered as a more exact statement of the relative number of oogonia produced on various mycelia of a given series than could be made in any other way.

TABLE IX

S. ferax. Part of Record from Set I Showing Agreement Between Count and Cross Count. Mycelia in 0.05 *Percent Haemoglobin. Temperature* 16°–18° *C. Figures to Nearest Whole Number*

1	2	3	4	5
		1st Count	Cross Count	Average
1	Pea	40	44	42
2	Peptone 1%	37	31	34
	" 1% duplicate	43	45	44
3	Peptone 0.5%	36	32	34
4	Peptone 0.2%	27	34	31
	" 0.2% duplicate	26	23	25
5	Peptone 0.1%	16	16	16
6	" + sucrose	19	17	18
7	" + maltose	43	38	41
8	" + sucrose + salts	27	28	28
9	Peptone + maltose + salts	44	40	42
10	Peptone 0.2% + sucrose	55	52	54
11	" 0.2% + maltose	45	50	48
12	" 0.2% + " + salts	64	47	56
13	" 0.1% + salts	32	33	33
14	" 0.2% + "	32	38	36

Record of Series I, October, 1914.—For this experiment the food solutions were contained in tubes each holding 50 cc. of liquid. These were inoculated and allowed to grow for seven days and transferred to 0.05 percent haemoglobin October 1, 1914. At the time the mycelium from one tube was transferred to haemoglobin, that from a duplicate was washed, dried and weighed, and a third tube was left for forty-eight days and the contents then washed, dried and weighed.

Table X gives the complete record of this test. Unfortunately a rather large number of sporangia were produced in the haemoglobin, especially by the mycelium from pea and from peptone 1 percent and this interferred materially with the production of oogonia. In the following discussion the record of these two will be disregarded as being probably too greatly affected by the production of sporangia.

TABLE X

Series I. Saprolegnia ferax. Mycelia Out of Media Under Column 2, *Transferred to* 0.05 *Percent Haemoglobin; Sugars and Salts* 1/100 *Molecular Except as Noted. Weight in Milligrams of Mycelia Out of* 50 *cc. Liquid. Record of Sporangia and Oogonia Produced. Figures for Number of Oogonia to Nearest Whole Number*

1	2	3 Weight, 7 Days.	4 Weight, 48 Days.	5 Sporangiain Water, 7 Hrs.	6 Sporangia in Water, 24 Hrs.	7 Sporangia, Haemoglobin, 3 Days.	8 Oogonia, 10 Days.
1	Pea	0.018	0.056	III	III	III, oogonia started	42
2	Peptone 0.1%	0.002	0.005	II	II–III	I+	16
3	" 0.2%	0.003	0.011	I–II	II	I+	30
4	" 0.5%	0.004	....	II+	II–III	I–II	34
5	" 1%	0.0036	0.037	I–II	II–III	II–III	36
6	" 0.1%, sucrose	0.0035	0.007	0–I	I+	0, oogonia started	18
6*m*	" 0.1%, maltose	0.0044	0.033	II	II–III	I	41
7	" 0.1%, sucrose and salts	0.0054	0.010	0–I	I+	I–II	28
7*m*	" 0.1%, maltose and salts	0.004	0.033	I	II–III	0–I	42
8	" 0.2%, sucrose	0.0054	0.013	0–I	II	0–I	53
8*m*	" 0.2%, maltose	0.005	0.036	0–I	II	II	43
9	" 0.2%, sucrose and salts	0.006	0.020	Few	I–II	II	51
9*m*	" 0.2%, maltose and salts	0.005	0.044	Few	II–III	I–II	50
10	" 0.1%, salts	0.002	0.0066	0	I–II	I	36
11	" 0.2%, "	0.0023	0.007	Few	I–II	I	28
12	" 0.2%, " 1/333 molecular	0.003	....	0	I–II	0	43

If these records are arranged in two groups, the first containing those in which 16–36 oogonia were found in 25 square millimeters, and the other those with 41–53 oogonia per square it will be noted that the first group contains those culture media without sugar, except for numbers 6 and 7, in which sugar was used. The second group includes all those with sugar except 6 and 7 and also one (12) to which no sugar was added. See Table XI.

The weights for the seven-day-old mycelia are, as a rule, higher in the second than in the first group and this is even more striking

TABLE XI

Series I. Saprolegnia ferax. Record of Table X Arranged in Two Groups According to the Number of Oogonia Per Square of 25 Square Millimeters. Weights in Milligrams

1 No.	2 Number of Oogonia	3 Weight in 7 Days	4 Weight in 48 Days	5 Increase in Weight
Group I:				
2	16	.002	.005	×2.5
6	18	.0035	.007	×2
7	28	.0054	.010	×1.85
11	28	.0023	.007	×3
3	30	.003	.011	×3.3
4	34	.004	——	—
10	36	.002	.0066	×3.3
Group II:				
6*m*	41	.0044	.033	×7.5
7*m*	42	.004	.033	×8
8*m*	43	.005	.036	×7
12	43	.003	——	—
9*m*	50	.005	.044	×9
9	51	.006	.020	×3.3
8	53	.0054	.013	×2.5

when the weights of the mycelia forty-eight days old are compared. Number 12 has the one disturbing record in this series. With a small weight, it combines a rather large number of oogonia. From the weight of mycelium as well as from the composition of the food, it was to be expected that this lot would appear in a group with numbers 2, 3, 10, and 11. The explanation of the peculiar behavior of this lot doubtless lies in the fact that no sporangia were produced in haemoglobin. As has already been shown, the suppression of sporangia, in a solution of this sort, reacts very favorably on the formation of oogonia, but why no sporangia were formed in this case it is impossible to say. A study of the record in Table XI would seem to show an agreement between weight of mycelium and the number of oogonia produced. But this is true only as showing a trend toward a minimum food requirement. In group I, for example, 0.1 percent peptone is clearly too poor a food to produce vigorous mycelium and the addition of sucrose alone does not add to the vigor of growth. When, however, salts are added, or higher concentrations of peptone used, the number of oogonia at once approaches that of the lower numbers in the second group. The separation of the groups along the line between 36 and 41 oogonia is, of course, arbitrary and only for the purpose of illustration. Differences as great as between 28 and 41 might well be found between two counts of the same dish, but no two duplicate dishes would, without contamination or other accident, show as great a difference as that between 28 and 53.

While the upward trend in the number of oogonia, with the increase in the food furnished, is evident, there is no corresponding increase in the weight of mycelium. It will be noted also that the amount of increase in the weight of mycelium between seven and forty-eight days is by no means uniform and a study of this feature brings out a rather interesting point. In Table XII these data are arranged in the order of the increase in weight of each mycelium due to the growth from the seventh to the forty-eighth day.

TABLE XII

Series I. S. ferax. Record of Table X Arranged in the Order of the Increase in Weight, Due to Growth Between Seven and Forty-eight Days

1	2	3	4	5	
Record Number	Amount Increase	Weight 7 Days	Weight 48 Days	Number of Oogonia	
7	×1.85	.0054	.010	28	Group I. Average number of oogonia 29. Average excluding No. 8—21.
6	×2	.0035	.007	18	
2	×2.5	.002	.005	16	
8	×2.5	.0054	.013	53	
11	×3	.0023	.007	28	Group II. Average number of oogonia 36.
10	×3.3	.002	.0066	36	
3	×3.3	.003	.011	30	
9	×3.3	.006	.020	51	
8*m*	×7	.005	.036	43	Group III. Average number of oogonia 47.
6*m*	×7.5	.0044	.033	41	
7*m*	×8	.004	.033	42	
9*m*	×9	.005	.044	50	

Of the first group, those showing an increase in weight of not more than 2.5 times, number 8 is the only one credited with a large number of oogonia; of the second group number 9 is credited with a much larger number of oogonia than the others. The third group differs strikingly in the much greater increase in weight shown by all the mycelia. Reference to Table X will show that the culture medium for all those in group III contained maltose, and that this group includes only those in which maltose was added to the culture medium.

The record of numbers 2 and 6 may be compared since in both cases 0.1 percent peptone was available, but sucrose had been added to solution 6. It is apparent that the fungus was not able to utilize sucrose in the presence of this strength of peptone while it made free use of maltose as shown by the record of number 6*m* which also contained 0.1 percent peptone but to which maltose had been added instead of sucrose. Numbers 8 and 9 contained the same amount of sucrose as 6 had and in addition 0.2 percent peptone, and 0.2 percent peptone and salts respectively, and on these mycelia were developed the largest number of oogonia of any lot in the series. This large number of oogonia cannot be accounted for by the higher percentage of peptone alone nor by the presence of salts alone, as then numbers 3 and 11 should have given comparable results. It is evident that in numbers 8 and 9 the sucrose was used but the weight record indicates the use of only a small part of it. During the first seven days' growth the fungus was very well nourished as shown by the fact that the weights at the end of that time are among the highest. This rate of growth, however, was not maintained as is shown by the small increase in weight. The explanation is doubtless that in numbers 8 and 9 part of the sucrose was changed to invert sugar owing either to too prolonged heating at high pressure while sterilizing, or to the presence of salts in the solution together with a high temperature. If this is true the fungus would find invert sugar present as soon as new growth began; development would then be rapid in the next few days or as long as the invert sugar lasted. Upon the exhaustion of the invert sugar growth would slow down and the final weight would be less than that from solutions containing more available carbohydrates than sucrose. Further, if the fungus were removed before the invert sugar present was consumed it would be in the same physiological condition as it would have been if all the carbohydrate consisted of invert sugar.

These theoretical considerations are supported by the facts of the experiment. After seven days numbers 8 and 9 had made as large a growth as any lot, but at the end of forty-eight days the total increase was 2.5 times the weight at the end of seven days, as compared with 7–9 times the weight in the case of 8*m* and 9*m*. Growth was at first rapid, then slowed down; the invert sugar was used up. That the mycelium at the end of seven days was in good condition is shown by the large number of oogonia produced.

At the end of seven days and while the fungus in solutions 8 and 9 was in the full enjoyment of an abundant supply of invert sugar the mycelium was transferred to haemoglobin; that this mycelium was then in good condition is shown by the large number of oogonia produced. We may then conclude that invert sugar is an especially valuable food for the subsequent production of oogonia; that sucrose cannot be utilized by this fungus, but that maltose is used and is a good food both for vegetative growth and for the production of oogonia.

(In series II the oogonia rarely developed oospores. This was due probably to contaminated haemoglobin. The entire set was discarded.)

Series III.—This series was prepared to repeat the work in series I and also to determine if possible which of the two sugars present in sucrose, dextrose or levulose, was most used by the fungus. Solutions containing salts as well as sugars were also prepared and cultures grown in them, but since the large number of cultures thus set up would make it impracticable to handle all at one time those with salts were transferred in a separate lot. For some reason not determined, all of the mycelia from the solutions containing salts failed to make oogonia or at most produced but a few oogonia each. Since this result was obviously due to some abnormal condition the entire lot was discarded and the results with the sugars alone are presented.

Table XIII gives all the data for this series.

Table XIII

Series III. Saprolegnia ferax. Mycelium Out of Culture Media in Column Two. Grown Seven Days Before Transfer to 0.075 *Percent Haemoglobin and Thirty Days in* 200 *cc. Liquid Before Weighing; Weights in Milligrams. Oogonia Count Recorded to Nearest Whole Number. Temperature* 15°–18° C. *Sugars All* 1/50 *Molecular*

No.	Food	Weight	Record After 2 Days		Oogonia After	
			Sporangia	Oogonia	3 Days	17 Days
1	Pea	0.041	I+	II	III	46
2	Peptone 0.1%	0.0172	0–I	0	Few	21
3	“ 0.2%	0.0376	I	0–I	I	46
4	“ 0.5%	0.079	II	0	0–I	38
5	“ 1%	0.124	II	0	I	41
6	“ 0.1% + sucrose	0.024	I–II	0	0–I	29
7	“ 0.1% + maltose	0.068	II	0	I	65
8	“ 0.1% + dextrose	0.023	II	0	0–I	28
9	“ 0.1% + levulose	0.061	I	I	II–III	73
10	“ 0.2% + sucrose	0.0404	II	0	I	28
11	“ 0.2% + maltose	0.099	II	I	II	43
12	“ 0.2% + dextrose	0.0474	II	0	Few	24
13	“ 0.2% + levulose	0.065	II	Starting	0–I	55
20	Haemoglobin 0.1%	0.0199	I–II	0	Few	22

It will be noted that not so many sporangia were formed in the 0.075 percent haemoglobin as in the 0.05 percent used in series I, and that the numbers of sporangia were nearly the same in all the dishes, so that any influence on the production of oogonia can be disregarded. This table shows, as did Table X, that there is no constant relation between the amount of dry weight a culture medium will produce and the number of oogonia formed by the mycelium after transfer. In only one case do these figures agree; the mycelium out of 0.1 percent peptone is at the bottom of the list in both instances. The greatest weight was made by mycelium out of 1 percent peptone which was seventh in the production of oogonia. Mycelium out of solution number 9 had the largest number of oogonia and was sixth in weight. The second heaviest mass of mycelium was out of solution number 11 and this was sixth in the production of oogonia. The results secured in the first experiment are therefore confirmed so far as concerns this point. The value of maltose is also confirmed, and the great difference between the value of dextrose and levulose in the conditions of this experiment is brought out. Dextrose added to peptone increased the weight of mycelium slightly, but the number of oogonia not at all, while the addition of levulose produced a mycelium with a tendency toward the production of oogonia quite out of proportion to its value for vegetative growth.

In the course of this work many tests were made to determine the effect on the production of oogonia when salts or sugars were added to the haemoglobin. If it is true that levulose is an especially favorable form of carbohydrate for the production of oogonia, this fact should also be brought out if levulose is added to haemoglobin. This was done in a number of cases and the results confirm the conclusion that for *S. ferax* levulose has an especial value for the production of oogonia.

In Table XIV the results of one test are brought together.

TABLE XIV

Saprolegnia ferax. Mycelium Out of Pea Extract in Haemoglobin and in 0.025 *Percent Haemoglobin and sugars. In Every Case Concentration of Sugar Equals* 1/200 *Molecular. Figures Represent the Number of Oogonia Formed in* 25 *Sq. Mm. of Mycelium After Nine Days*

Haemoglobin 0.025%	Haemoglobin 0.05%	Haemoglobin + Dextrose	Haemoglobin + Levulose	Haemoglobin + Maltose	Haemoglobin + Sucrose
21	34	49	129	14	92

It will be noted that here sucrose has also had a marked effect, due doubtless, as before suggested, to the inversion of a part of the sucrose. In dextrose the growth was short and dense but did not fill the dish, while in maltose the growth was very dense and rapidly filled the dish with a mass of delicate hyphae. Possibly a lower concentration of maltose might have given better results. The growth in the haemoglobin-levulose solution was open but vigorous; oogonia appeared earlier than in the other dishes and the number formed was so much greater than the number produced by mycelium of the same origin in haemoglobin alone or in haemoglobin and dextrose that there can be no mistaking the conclusions that are indicated. The fact that these results confirm those secured when levulose was offered as a food during vegetative growth only makes them especially significant.

The results with *S. ferax* support the general conclusions arrived at in the experiments on *A. racemosa*. The weight of mycelium secured from any culture is no measure of the number of oogonia which that mycelium may be expected to produce when transferred to a suitable environment. There is however a minimum concentration of food at and below which both the weight of the dry matter and the number of oogonia decrease. This minimum is about at 0.1 percent peptone for the production of both sporangia and oogonia.

Levulose is especially valuable as a food for the formation of oogonia; dextrose is not used as freely and sucrose not at all unless previously inverted by other agencies, while maltose is readily used.

Experiments with S. monoica

S. monoica was used because it normally has an antheridium on each oogonium and oogonia are freely produced, even under somewhat adverse conditions. This species is closely related to *S. ferax*, and experiments with it might be expected to confirm the conclusions drawn from the results of previous experiments. It was also thought that some light might be thrown upon the kind of food that is best for the production of antheridia. Cultures were made in flasks containing 200 cc. of liquid in the same manner as for *S. ferax* series III; after growing five days these cultures were transferred to 0.05 percent haemoglobin.

Table XV gives the complete record for this set.

Table XV

Series VIII. S. monoica. Mycelium From Media Under Column 2, Transferred to 0.05 *Percent Haemoglobin. Weight of Mycelium in Milligrams after Thirty Days' Growth in* 200 *cc. Liquid. In Numbers* 6–17 *the Amount of Peptone is Always* 0.1 *Percent, of Sugar* 1/50 *Molecular, and of Salts* 1/200 *Molecular*

No.	Food	Weight, Milligram	Sporangia 2 Days	Oogonia 10 Days	Antheridia, Percent	Weight of Mycelium Out of Haemoglobin, Milligram
1	Pea..................	.0398	II	80	21	.0157
2	Peptone 0.1%........	.0146	I	170	6	Lost
3	" 0.2%........	.0256	I	146	9	.0109
4	" 0.5%........	.0561	I–II	79	9.5	.0132
5	" 1%..........	.0863	II	108	7	.0138
6	" + sucrose...	.015	II	62	6	.0120
7	" + maltose...	.0598	II	58	12	.0144
8	" + dextrose...	.0281	II	96	10	.0128
9	" + levulose...	.0581	II	119	20	.0152
14	" + sucrose + salts......	.3645	II	79	6	.0191
15	" + maltose + salts......	.2151	0–I	52	15	.0219
16	" + dextrose + salts......	.1810	II	105	13	.0188
17	" + levulose... + salts......	.2100	II	103	64	.0186

A striking feature of this record is the very large number of oogonia found on the mycelium from 0.1 percent and from 0.2 percent peptone. The preliminary tests showed that in this species mycelium from 0.1 percent peptone produced as many oogonia as that from 1 percent but such a large number as recorded in Table XV was quite unexpected. In this series the tests in haemoglobin were conducted in quadruplicate. Before making the final records all dishes were carefully examined and the number of oogonia in each was found to be practically the same. Probably the large number of oogonia is connected with the smaller number of sporangia in these lots, for, as has been said, a mycelium producing few sporangia is in better condition to produce oogonia than one that has produced many sporangia. This fact introduces an element of uncertainty into this work that is always present and must be taken into consideration in interpreting the results. Leaving the record of numbers 2 and 3 out of consideration it is apparent that levulose is a food favorable for the formation of oogonia out of proportion to its value for vegetative growth, and that sucrose with 0.1 percent peptone alone is probably not available to the plant.

The weight of mycelium out of solution number 6 is practically the same as that out of number 2. When salts are added to peptone and sucrose the number of oogonia produced is materially increased and the weight is much greater than from solutions without salts. As before suggested, this is probably due to the formation of invert sugar from the sucrose by the action of high temperature and salts.

There is evidently no relation between weight of mycelium and number of oogonia even when the record of numbers 2 and 3 is discarded. The weight of mycelium was about equal out of solutions 4, 7 and 9 but the number of oogonia varied from 58 on mycelium out of solution 7 to 119 out of solution 9. Again, mycelia out of solutions 15 and 17 show practically the same weight but vary in the number of oogonia recorded from 52 to 103. For vegetative growth *S. monoica* does not use dextrose as freely as it does maltose and levulose, agreeing in this with *S. ferax*. The difference in weight is striking and appears consistently in all the records, but it is doubtful whether it is safe to draw more than the most general conclusions from the record of the number of oogonia, since this species produces oogonia more readily and abundantly than any other species the writer has isolated. In general, however, the conclusions previously drawn seem warranted by the results of this experiment also. The minimum food required for an abundant supply of oogonia is less than that demanded by *S. ferax*, the critical concentration evidently being below 0.1 percent peptone, but there is no uniform relation between weight of mycelium and number of oogonia. The value of levulose is also confirmed.

Klebs found that when *S. mixta* was placed in 0.05 percent haemoglobin oogonia were readily formed but they were not accompanied by antheridia. With the same treatment *S. monoica* gave similar results although some oogonia with antheridia were always present. The number of oogonia accompanied by antheridia was determined by counting 100 or 200 or sometimes a larger number of oogonia under a 16 millimeter objective and recording the number to which antheridia were attached. While the figures cannot be considered final there is a consistent trend toward an increase in the number of antheridia when the culture medium has contained a form of invert sugar, especially levulose. Number 14 is the only exception to this. In this solution much of the sucrose was probably converted into invert sugar and if so the number of antheridia should approach that in the case of the solutions with invert sugar. The larger number of antheridia present on mycelium grown in the presence of levulose is especially interesting, since when this sugar is added to haemoglobin the resulting growth produces oogonia nearly all of which are accompanied by antheridia, while when dextrose is substituted for levulose the oogonia are larger but only a few of them have antheridia.

In this series mycelium from each culture solution was transferred to four dishes of haemoglobin and especial care was taken to have these transferred masses of as nearly the same size as possible, as it was intended to weigh the mycelium from the haemoglobin after the final oogonium record. This was done to see whether there was any relation between the number of oogonia and mass of mycelium produced in the haemoglobin. The column at the right in Table XV shows the total weight of the mycelium in the four dishes. It is true that slight differences in the masses originally placed in the haemoglobin probably did exist but when it is remembered that the figures represent the total weight from four dishes after several days growth, it will be seen that the small piece of mycelium originally put into the haemoglobin could in no case have exceeded one milligram in weight. The difference in weight between any two such masses therefore would be represented at most by one unit in the fourth decimal place and could have no influence on the general result. Here again the evidence confirms the conclusion previously reached that there is no relation between the weight of mycelium and the number of oogonia produced by it. On March first another series was set up with *S. monoica* to confirm the results obtained by series VIII. Peptone and sugars only were used and the resulting data are presented in Table XVI. The cultures in this series were made in Joseph Kavalier Bohemian glass flasks.

TABLE XVI

Series IX. S. monoica. Mycelia Out of Solutions Under Column 2. Transferred to 0.05 *Percent Haemoglobin After Five Days. Weighed After Thirty Days in* 200 *cc. Solution. Weight in Milligrams. Number of Oogonia in* 25 *Square Millimeters. Sugars All* 1/50 *Molecular*

1	2	3	4	5
	Media	Weight in Milligrams	Sporangia 3 Days	Oogonia 8 Days
1	Peptone 1%	.0980	0	89
2	" 0.1%	.0244	A few	48
3	" 0.1% + dextrose	.0270	A few	52
4	" 0.1% + levulose	.0196	A few	85
5	" 0.1% + maltose	.0773	A few	57
6	" 0.1% + sucrose	.02675	A few	45

In this series the small weight of the mycelium out of peptone-levulose is difficult to account for. Unfortunately the duplicate flask failed of inoculation, and the weight represents but one culture instead of an average of two as in the other cases. The number of oogonia on the mycelium out of levulose-peptone was however again greater than that on mycelia out of other solutions containing sugars and was practically the same as that out of 1 percent peptone. Maltose was freely used for vegetative increase but the mycelium out of maltose-peptone did not develop a large number of oogonia.

At the same time that the cultures recorded in Table XVI were set up, another series was planned to determine what effect salts added to the peptone alone, or to peptone and sugars, would have on the vegetative growth or on the number of oogonia. The time available was insufficient for repeating this experiment and the results are offered as suggestive rather than as proof of the conclusions that seem to be warranted.

Table XVII gives the results of this experiment. The mycelia out of the solutions marked with an asterisk were overheated during the experiment, and the number of oogonia formed cannot be regarded as fairly representing the capacity of each mycelium to produce oogonia. The figures have, therefore, been omitted.

It would appear from the record that the addition of phosphate salts, and, to a much less degree, potassium nitrate, stimulates growth while calcium nitrate, potassium sulphate, and magnesium sulphate have no marked effect. The only conclusion that seems fully justified by the somewhat uncertain results of one trial, however, is that phosphates exert a marked effect both on the amount of dry matter and on the number of oogonia produced. Comparing numbers 5 to 10 with 16 and 17 it will be seen that while in the latter the use of phosphates with peptone alone has increased the weight materially over that when peptone only was used (21), it still falls short of the weight of the mycelia out of the solutions containing sugar, peptone and phosphates. The number of oogonia produced on the various mycelia is, however, of the same order of magnitude in all cases, in which a phosphate was used, and this number is much larger than that obtained when other salts were used (compare 19 and 20).

The conclusions seem warranted, therefore, that in the case of *S. monoica* also there is no necessary relation between weight of mycelium and the number of oogonia that may be produced; that levulose is especially adapted to the production of oogonia and that phosphate salts influence the number of oogonia produced to a greater degree than they do the increase in weight of mycelium.

Table XVII

Series IX. S. monoica. Mycelia Out of Culture Media in Column Two, Transferred to 0.05 *Percent Haemoglobin After Seven Days' Growth, and Weighed After Growing Thirty Days in* 200 *cc. Solution. Weights in Milligrams. Sugars All* 1/50 *and Salts All* 1/200 *Molecular; Peptone* 0.1 *Percent*

1 No.	2 Media	3 Weight	4 Sporangia	5 Oogonia
1	Peptone + dextrose + KNO_3	.0423	0	41, oogonia small
2	" + dextrose + $CaNO_3$	.0281	0	80
3	" + levulose + KNO_3	.03675	0–1	93
4	" + levulose + $CaNO_3$	.02095	0–1	40
5	" + dextrose + KH_2PO_4	.2011	0–1	94
6	" + levulose + KH_2PO_4	.2084	0	79, large and well filled
7	" + dextrose + NaH_2PO_4	.13835	0	90, many small ones
8	" + levulose + NaH_2PO_4	.17145	0–1	89, mostly small ones
9	" + dextrose + $Ca(H_2PO_4)_2$	.15954	0–1	110
10	" + levulose + $Ca(H_2PO_4)_2$	.1093	1	93, large well filled
11	" + dextrose + KSO_4	.0291	0–1	*
12	" + levulose + KSO_4	.01855	Few	*
13	" + dextrose + $MgSO_4$	.0404	"	*
14	" + levulose + $MgSO_4$	.02225	0–1	54
15	" + KNO_3	.0183	Few	*
16	" + KH_2PO_4	.0426		111
17	" + NaH_2PO_4	.0401		101, small
18	" + $Ca(H_2PO_4)_2$	.0416		*
19	" + KSO_4	.0213		68
20	" + $MgSO_4$	.0276		57, small
21	" 0.1%	.0244		48

Experiments with Achlya prolifera

Several sets of experiments were carried on with this species along the same lines as those before described. The results however were not satisfactory but are presented because so far as they may be accepted at all they seem to confirm most of the conclusions arrived at by a study of the previously considered species. The oogonia could not be counted as the mycelium was so thickly matted that many oogonia could be but dimly seen and the distribution of the oogonia was often so irregular that it was not possible to secure a fair average. The number of the oogonia was therefore estimated. The most important cause of the lack of good results with this species is that no entirely satisfactory solution was found to which the mycelium could be transferred for the formation of oogonia. Early experiments showed that haemoglobin was worthless, oogonia were formed but no oospores were ever developed. Many experiments were then made with peptone and sugars as suggested by Obel ('10) and also by placing masses of mycelium into pure water or water to which salts had been added.

A. prolifera will form oogonia in water as is the case with *A. racemosa*, but unfortunately the formation of oogonia is prevented by a temperature lower than that at which sporangia continue to be freely formed. If however a mass of mycelium is left in the water in which it was placed on being taken from the culture flask, it may find sufficient food present to prevent any free formation of sporangia and thus permit the production of oogonia. This method was therefore used with the others, but the results have not been uniform. Many solutions containing peptone and sugars were tried and at temperatures from 10° to 24° C. A few of the results of these tests are given in Table XVIII; it is thought unnecessary to extend the record as in most of the other cases only vegetative growth took place with an occasional oogonium, sometimes with and sometimes without oospores.

Oogonia were formed under most of the conditions presented in the table but they were seldom well filled, often only a small number of the oogonia containing oospores. The failure to produce oospores appeared generally to be connected with excessive vegetative growth although the specific value of the carbohydrate is clearly seen when the record of numbers 3, 4, 6 and 7, each of which contained 0.01 percent peptone, are compared, or when the record of number 8 is compared with that of number 11. Both of these solutions contained 0.02 percent peptone but when this only was present the fungus made a dense growth which rapidly filled the dish and no oogonia were produced. The addition of sucrose restricted the growth and caused the development of many well filled oogonia In nearly all peptone-sucrose solutions oogonia were freely produced; maltose and dextrose were also generally satisfactory; levulose always encouraged a great growth of delicate hyphae while few oogonia were formed and those were usually empty.

TABLE XVIII

Achlya prolifera. Mycelia Out of Pea and Peptone-Sucrose-Salts Solution into Solutions Under Column 2. Estimated Number of Oogonia and Oospores. The Figures for Oospores at Temperatures Given are Estimated Percentages

1	2	Peptone-Sucrose-Salts, 20°–22° C.		10°–15° C.		Pea Extract, 20°–22° C.		Pea Extract, 16°–18° C.	
		3	4	5	6	7	8	9	10
No	Media.	Oog.	Oosp.	Oog.	Oosp.	Oog.	Oosp.	Oog	Oosp.
1	Haemoglobin 0.1%	Few	0	0–I	0				
2	" 0.05%	I–II	0	II	0				
3	Peptone 0.01%....					0–I	Few	I	Few
4	Peptone 0.01% + dextrose M/500					I–II	"	II–III	50
5	Peptone 0.01% + levulose M/500					0–I	"	I–II	Few
6	Peptone 0.01% + maltose M/500					II–III	50	III	50
7	Peptone 0.01% + sucrose M/200					II–III	50	III	90
8	Peptone 0.02%....					Growth only		Great growth only	
9	Peptone 0.02% + dextrose M/1000					I–II	25	I	0
10	Peptone 0.02% + levulose M/250					I	Few		
11	Peptone 0.02% + sucrose M/300					III	95	II	25
12	Peptone 0.02% + sucrose M/500					Great growth only		Great growth only	
13	Peptone 0.03% + dextrose M/300	I	50	I–II	Few				
14	Peptone 0.03% + maltose M/300	II–III	60	0–I	0				
15	Peptone 0.03% + sucrose M/300	III	95	II	50				

The experiment with 0.02 percent peptone and sucrose, number 11, was among the earlier ones and it seemed reasonable to conclude that this was a favorable medium for the production of oogonia and that experiments at room temperature would give reliable results. Series V was therefore planned and carried through, the mycelia being grown in the culture media for seven days and then transferred, after being twice washed, to a solution containing 0.02 percent peptone and 1/300 molecular sucrose. The entire experiment was carried through at room temperature. The results are presented in Table XIX. In all cases more or less growth took place in the peptone-sucrose solution and in some the formation of oogonia and oospores was all that could be desired. The results, as a whole, however, are not regular enough to warrant the statement that the failure of some mycelia to produce oogonia was due to the preceding conditions of growth. This however does not affect the general value of the results as pointing to the conclusions previously reached. It is true that the mycelia with the largest number of oogonia and oospores are also among those having the greatest weight, but not all of the latter class have developed large numbers of oogonia (see numbers 12, 15, 17), while the records of several numbers show a good development of oogonia together with weights one half to one third as great as those from other lots. When the solutions containing sucrose are not overheated there is clearly little invert sugar; the fungus makes practically no more growth in such solutions than in peptone alone and the number of oogonia is about the same on mycelia from all these solutions; compare the record of numbers 2 and 6, 3 and 10. The weight of mycelium out of levulose-peptone solution was nearly the same as that out of peptone alone and the mycelium out of the former solution responded to the peptone-sucrose environment by an unusually vigorous growth of delicate hyphae which soon filled the dish. This phenomenon was observed in two out of three series of experiments while in a third oogonia were formed on mycelium out of levulose-peptone but these were not nearly so numerous as on other mycelia. The influence of levulose when used as a culture medium for *Achlya prolifera* was certainly against the formation of oogonia. The addition of salts produced a marked increase in growth with all the sugars, but most with the sucrose, doubtless because of the extensive inversion of the sucrose under the action of the high temperature and salts.

Two other series of experiments were made with *A. prolifera*, and in both the results were similar to those recorded for series V. In one series a transfer solution weaker in peptone (0.01 percent) and stronger in sucrose (1/200 molecular) was used in the hope that this would lead to a better development of the oospores. The results were, however, no more uniform than before as only a small proportion of oogonia in any lot contained oospores. In another series various salts were added to the peptone-sugar solutions and to the peptone alone. The salts used were various phosphates, nitrates and sulphates, the same as were used in the experiments with *S. monoica*,

TABLE XIX

Series V. Achlya prolifera. Mycelia Grown Seven Days in Media Shown in Column 2, Transferred to Peptone 0.02 *Percent + Sucrose M/*300. *Weight of Mycelia Grown Fifteen Days in* 200 *cc. of Media Shown in Column 3. Numbers of Oogonia and of Oogonia with Oospores Estimated, the Latter in Percentages*

1	2	3	4	5
		Weight in Milligrams	Oogonia	Oogonia with Oospores
1	Pea	.091	II	25%
2	Peptone 0.1%	.025	II	10 "
3	" 0.2%	.0332	II	10 "
4	" 0.5%	.039	II–III	20 "
5	" 1.0%	.0405	II–III	75 "
6	" 0.1% + sucrose	.0270	II	10 "
7	" 0.1% + maltose	.0476	II	5 "
8	" 0.1% + dextrose	.036	0–I	Few
9	" 0.1% + levulose	.0286	0	
10	" 0.2% + sucrose	.0324	I	Few
11	" 0.2% + maltose	.058	II	10%
12	" 0.2% + dextrose	.0412	I	Few
13	" 0.2% + levulose	.0346	0–I	"
14	" 0.1% + sucrose + salts	.1140	II–III	75%
15	" 0.1% + maltose + salts	.0950	I	Few
16	" 0.1% + dextrose + salts	.1234	II–III	90%
17	" 0.1% + levulose + salts	.085	I	40 "
18	" 0.1% + salts	.0466	II	Few
20	Haemoglobin 0.1	.0242	I	10%

see Table XVII. In every case the mycelia grown in solutions containing potassium, sodium or calcium phosphate, either with or without sugar, when transferred to peptone-sucrose solutions grew rapidly and filled the dish with a mass of fine hyphae on which few or no oogonia were formed. Mycelia out of solutions containing nitrates or sulphates, on the other hand, made little growth when transferred to the peptone-sucrose solution and the number of oogonia was recorded as I, II, or III on various mycelia. As in the case of *S. monoica*, the addition of phosphates to the solution increased the vegetative growth markedly, and the great growth without oogonia may be because the peptone-sucrose solution used was not the best for the production of oogonia by this kind of mycelium.

DISCUSSION

In the preceding pages attention has been called to such conclusions as seemed warranted by the record of each set of experiments.

These conclusions will be briefly discussed under the following heads.

1. The relation between the weight of mycelium and the number of oogonia produced.
2. The expression in one environment of tendencies acquired in a former environment.
3. The value of levulose.
4. The value of sucrose.
5. The value of phosphates.

The Relation between Weight of Mycelium and the Number of Oogonia Produced

The question which was stated at the beginning of the paper, whether any constant and necessary relation exists between the vegetative growth and the reproductive activity in several species of the Saprolegniaceae must be answered in the negative. The record of experiments with *Achlya racemosa*, *A. prolifera*, *Saprolegnia ferax* and *S. monoica* all agree in showing that a mycelium out of a relatively poor solution, measured by vegetative growth, may produce more or larger oogonia than are produced on a mycelium from a solution that is much better for vegetative growth. This has, of course, nothing to do with the well-known fact that after a mycelium has been transferred to a new solution, vegetative growth may prevent the formation of oogonia. When a mycelium of *S. ferax* is taken from pea extract and placed in a haemoglobin solution that is stronger than 0.1 percent, few if any oogonia are produced; vegetative growth is excessive and prevents reproduction. Strong vegetative growth and reproduction cannot go on at the same time in the same solution but the problem under discussion is the relation between vegetative growth in one solution and reproduction by such a mycelium when transferred to another solution. It might be expected, a priori, that if a vigorous mycelium will produce a given number of oogonia when transferred to a suitable medium a more vigorous one, that is, one that had made a greater weight of dry matter in a given time in a fixed quantity of solution, would produce a greater number of oogonia. To produce oogonia after being transferred the fungus must draw upon its reserve materials and it might be thought that the dry weight of the mycelium would serve as a measure of the reserve materials available for the production of oogonia; but this was found not to be the case.

When only one food, peptone, was offered in varying concentrations there was a steady increase in the dry weight with the increase in the concentration of peptone but the mycelium out of 0.2 percent, 0.5 percent and 1 percent produced practically the same number of oogonia. See Series II, Table XIII and Series VIII, Table XV. When sugars or sugars and salts were added to the solution the absence of any fixed relation between weight of mycelium and the number of oogonia was even more marked. This does not mean, however, that a mycelium need not be vigorous in the sense in which this term seems to have been used by Klebs, Kauffman and Obel. They evidently meant that a mycelium must have been well enough nourished, and that a poorly nourished mycelium was to be guarded against in experimental work. It is very clear that a fungus can grow in a solution that will nourish too little to enable it to reproduce well; such a solution is to be considered as being below the minimum concentration necessary for satisfactory growth. For *S. ferax* a peptone solution containing 0.1 percent peptone, with or without sucrose provided none of the sucrose has been inverted, represents such a sub-minimum concentration. The minimum concentration for the production of a "well-nourished" mycelium may vary with the species. For *S. monoica* 0.1 percent peptone is enough to produce a well nourished mycelium if the number of oogonia produced be accepted as determining whether or not a mycelium has been well nourished, while *S. ferax* needs a stronger solution. Solutions containing nutrient substances at higher concentrations than the minimum will enable the fungus to produce an increased yield of mycelium but not a proportionate increase in the number of oogonia. Above the minimum concentration an increase in the number of oogonia will only be secured by changing the quality of the nutrient solution.

The Effect of a Given Environment May Not Become Evident Until the Plant Has Been Transferred to Another Environment

Nutritive substances do not, however, all have the same importance for the development of the different parts of the plant. Pfeffer ('97) states this in general terms when he says (Oxford Ed., 1900, p. 387) "the importance of a substance to a plant is not to be measured solely by the amount of growth which it induces."

While there is as yet no evidence that new species can be produced by changing the conditions, we can safely say that a plant is the creature of its environment in the sense that the potentialities of the species may be called forth in greater or less degree, or the expression of them may be retarded by the conditions under which it grows. The effect produced may be due to the environment as a whole or to certain factors. Klebs and others have already proved that conditions determine what phase of the plant's growth shall appear and it has been shown in the preceding pages that these phases may be influenced by the conditions prevailing during vegetative growth. The plant therefore not only responds to the immediate environment but is molded by it in ways that can find expression only after that environment has been changed for another.

While growing vegetatively a mycelium may acquire a tendency toward the development of sporangia and oogonia and such a tendency may be carried over to the new environment into which the mycelium is transferred. This fact was recognized by Klebs who said ('99, p. 585) that in all investigations on the production of sporangia the nutrition conditions of the mycelium have a specific importance as upon them depends the ability of the mycelium to respond to the change in nutrition by the formation of reproductive organs. Klebs further states that these two forms of reproduction make different demands on the protoplasm (Nährplasma), both quantitative and qualitative. So far as the present studies show, the minimum concentration of peptone needed to grow a mycelium that will develop sporangia or oogonia is about the same, but the addition of sugars favored the production of oogonia and that of salts the production of sporangia. In this connection reference may be made to some observations not given in the data above. Mycelium of *Achlya prolifera* grown in solutions of peptone and potassium nitrate, when transferred to water developed sporangia more freely than that from any other solution and the sporangia were strikingly large, with dark walls. A similar observation was made on *Dictyuchus monosporus*, mycelium of which from solutions with potassium nitrate developed large dark sporangia, while that out of solutions with mono-potassium phosphate formed small light colored sporangia. This phenomenon was observed a number of times and proved quite constant and characteristic.

The view that the fungus may, during vegetative growth, acquire a tendency toward the development of oogonia irrespective of the vigor of growth is strongly supported by the results obtained with levulose as a food as well as by those with phosphates. The fact that the effect of both levulose and phosphates was different on *Achlya prolifera*, from what it was on *Saprolegnia* serves to support this view and to show that the specific characters of different plants will react differently to similar environmental conditions. The close relationship existing between *S. ferax* and *S. monoica* made it reasonable to expect that similar responses would be made by these species to like conditions. But *Achlya* is, in habit and in choice of habitat, very different. As Petersen ('10) has pointed out, the Achlyas, though growing on dead animal matter, are frequently found on decaying plant parts, habitats not affected by the Saprolegnias.

It seems probable that the specific response of the plants grown in levulose and in phosphates may be due to a storage of reserve materials which are drawn upon as soon as the fungus finds itself in a poorer nutrient medium. Klebs suggests this but seems also to hint at a more intimate change in the character of the plasma. It is sufficient for the present purpose to point out the fact that the specific effect of one solution may be to awaken an appropriate reaction when the mycelium is transferred to another solution.

The Value of Levulose

The results of the experiments on both *Saprolegnia ferax* and *S. monoica* support the statement that levulose is used more readily by these species than other sugars except maltose and that it has a much greater effect in developing a tendency toward the production of oogonia than maltose has. This effect on the vegetating mycelium is confirmed in the case of both maltose and levulose by the effect of dilute solutions into which mycelia are transferred for the production of oogonia. In this case also maltose promotes vegetative growth to the almost entire exclusion of oogonia while levulose encourages the production of both oogonia and antheridia. The remarkable effect of levulose on some species was shown in the case of a still undescribed form which was collected as number 74. The details of the experiments with this form will be given in another paper but here it may be said that while, during almost two years of cultivation on natural and synthetic media, no oogonia were produced, these appeared when a mixture of leucin and levulose was used. The result was undoubtedly due to the levulose, as leucin alone or with any other carbohydrate or with salts failed to produce a like effect.

An apparent exception to the statement that levulose is as good a food for vegetative growth as maltose is found in the results shown in Table XVI. At the completion of this series (IX) it seemed difficult to account for the small amount of dry weight out of the peptone-levulose solution compared with that out of peptone-dextrose and peptone-maltose. On comparison of the conditions under which the various series of experiments had been made, it developed that the levulose used in the earlier experiments had been from a certain lot from Kahlbaum. The supply being exhausted and no more from the same source being available, the solutions for series IX were made up with levulose from Schuckardt. It seemed probable therefore that the smaller weight could be accounted for by the difference in the quality of the levulose. To test this the small amount remaining in the original Kahlbaum bottle was carefully collected and two lots of two times 100 cc. each of a solution containing 0.1 percent peptone and 1/50 molecular levulose were made up, one set with the Kahlbaum and one with Schuckardt levulose. After being inoculated with *S. monoica* the solutions were allowed to stand 20 days and the mycelia were then dried and weighed. The average weight of one lot out of the Kahlbaum levulose was .0212 milligrams, and out of the Schuckardt lot .0082 milligrams; practically the same degree of difference as that existing between the weights of mycelia out of peptone-levulose solutions recorded in Tables XV and XVI. It was not possible to undertake an analysis of the sugars from these two sources but the organism clearly recognized a difference between them. This experience perhaps suggests one of the reasons for the sometimes contradictory results secured by different workers. The reactions of living things to their surroundings are extremely delicate. Not only must the inherent characters of different species be considered, but in each species there are most probably a number of strains each of which makes its own peculiar response to a given set of conditions. Add to this the effects produced on a certain organism by supposedly chemically pure substances from different manufacturers and the conditions are present for varying results from which conclusions may be drawn leading to endless controversy. Possibly the result which Klebs reported, that *S. mixta* made a better growth in sucrose, dextrose and maltose than in levulose may have been due to the use of a poorer levulose than that which the writer used in the earlier series.

The Value of Sucrose

The records of the tests with solutions containing sucrose have been difficult of interpretation throughout the work. Under the discussion of Table X it was pointed out that the close agreement in the weight between the mycelium out of 0.1 percent peptone and that out of peptone and sucrose, as compared with the results when maltose was used, showed that little if any sucrose was utilized by the plant. The mycelium out of solution 8, however, showed not only a considerable increase in weight, but also in the number of oogonia formed over that out of No. 11, the difference between the two solutions being the addition of sucrose in number 8.

The results recorded in Table XIII are similar, the fungus growing in a solution containing sucrose having made little more weight than that grown in peptone alone, and the number of oogonia formed on the mycelium out of sucrose and peptone is nearly the same as that from the mycelium out of peptone alone. At the time the experiments were carried on the writer was not aware of the work of Noel Deerr ('10) on the effect of high temperatures on the inversion of sucrose. Deerr found that when sugar solutions in pure water were heated in an autoclave to a temperature of 110° no invert sugar was formed but that above that temperature a rapid increase in inversion with equal increments of temperature took place. Salts, as the nitrates of potassium, sodium and others, and the sulphates, as that of magnesium, increased the amount of inversion. The above facts offer an explanation of the results obtained with sucrose. The solutions used were always sterilized in an autoclave at a temperature of about 112°–120° C., but occasionally the temperature rose to 125° C. According to Deerr's results a small amount of invert sugar should have been made in practically every solution in which sucrose was used and it will be noted that the weights secured from mycelia out of peptone and sucrose are consistently a trifle greater than those from mycelia out of peptone alone. When salts were added, and occasionally when peptone and sucrose alone were present, a more marked increase in weight resulted.

In order to determine whether invert sugar was formed under the conditions prevailing in the above described experiments a series of solutions was made up containing in each case 0.1 percent peptone and 1/50 molecular sucrose. One set was sterilized at 110° C., one at 115° C. and the other at 125° C. Another series was also prepared in which the following salts were added to the peptone and sugar, magnesium sulphate, potassium nitrate, and di-hydrogen potassium phosphate, each salt being present in 1/600 molecular. A third series was like the second except that mono-hydrogen potassium phosphate was substituted for the di-hydrogen. The two series containing salts were sterilized at 110°, 115°, and 125° C. These solutions and temperatures were selected because they represent the conditions of the foregoing experiments and not for the purpose of making a detailed study of the reactions involved; quantitative determinations of the amount of invert sugar present in each lot after being sterilized for 30 minutes were made by Mr. R. B. Harvey, PhC. Of the solutions sterilized at 110° C. those with peptone and sugar alone and with the mono-hydrogen phosphate showed no inversion, while in that with the di-hydrogen phosphate 1.2 percent of the sucrose had been inverted. Sterilized at 115° C. the peptone-sugar solution and the solution containing mono-hydrogen potassium phosphate showed a trace of inversion, not enough to express in percentage, while in that containing the di-hydrogen 1.9 percent of the sucrose had been inverted. When the temperature at which the solution was sterilized went up to 125° C. 1.2 percent of the sucrose had been inverted in the solution containing peptone and sucrose only, 0.6 percent in that containing mono-hydrogen potassium phosphate, and 4.7 percent in that with di-hydrogen potassium phosphate. The fact that more inversion took place when the di-hydrogen potassium phosphate was used is interesting because this was used in practically all of the solutions made up for the experiments with Saprolegnias. On the whole the results correspond to those obtained by Deerr: at 110° C. there was no inversion, while at the higher temperatures an increasing amount of sucrose was inverted and when salts, including the di-hydrogen potassium phosphate were used a considerable amount of invert sugar was present before the solution was inoculated. Invert sugar was therefore available but varied in amount in different solutions and under different conditions.

We may conclude that *S. ferax* does not contain invertase and cannot make use of sucrose. In sterilized culture media, however, there is sure to be more or less invert sugar present owing to the inversion caused by heating and by the presence of salts. What has been shown to be true of *S. ferax* is equally true of the other Saprolegniaceae so far as they have been studied by the writer.

The Value of Phosphates

No attempt was made to determine the need of the fungus for inorganic nutriment. This would have been difficult, if not impossible, with peptone as the source of nitrogen, as the peptone itself contains a large percentage of total salts. An analysis of the Witte peptone used showed 2.78 percent total salts and a qualitative test revealed the presence of phosphates, sulphates, chlorides, and of potassium, sodium, magnesium, calcium, aluminum, and of ferric iron ($K_3(FeCu)_6$), so that it was manifestly impossible to exclude the presence of all of these salts except the one wanted for a particular experiment. However, the tests in which certain salts were added to the culture medium showed that with an abundant supply of phosphates *Saprolegnia* developed a greater capacity for the production of oogonia than when other salts were added, and this irrespective of whether potassium, sodium, or calcium phosphate were used. This result is in general agreement with the conclusions of Klebs, that the phosphates favored the production of oogonia, though Kauffman secured as good results from some of the other salts as from the phosphates.

In *Achlya prolifera* the increase in growth due to the phosphates was marked, but in this case the mycelium may have carried over sufficient reserve material to produce a vigorous growth in the peptone-sucrose solution, and thus prevented the formation of oogonia.

SUMMARY

1. There is no constant and necessary relation between vegetative growth and sexual reproduction when the food offered exceeds the minimum concentration necessary for the given species.

2. The minimum concentration of food necessary varies with the species but lies, in general, in the neighborhood of 0.1 percent peptone for the production of both sporangia and oogonia.

3. While growing vegetatively a mycelium may develop tendencies that may affect the number and character of the reproductive organs produced under subsequent and different conditions.

4. Of the carbohydrates used maltose and levulose are especially useful for vegetative growth and the latter has an especial value for the production of oogonia.

5. Sucrose is probably not used by species of Saprolegnia or of Achlya unless it is first inverted by some other agency.

6. Phosphates in the culture solution tend to increase the reproductive capacity of the fungus.

7. In preparing a culture solution for the Saprolegniaceae due regard must be paid to the fact that the conditions present during vegetative growth may affect the mycelium qualitatively as well as quantitatively. Not only is a vigorous mycelium needed but the composition of the medium must be accurately given for each experiment if workers are to arrive at comparable results.

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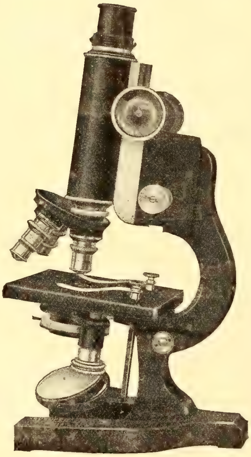

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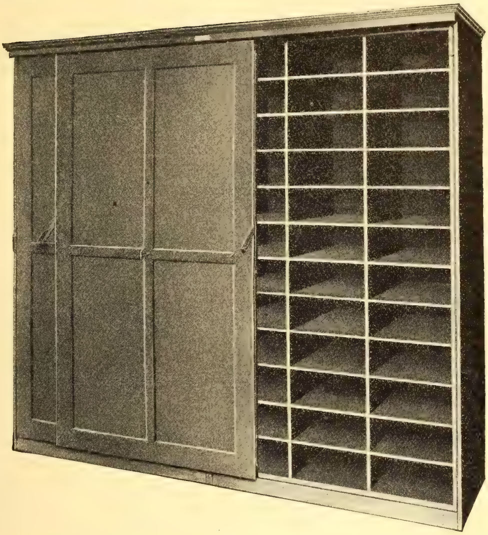

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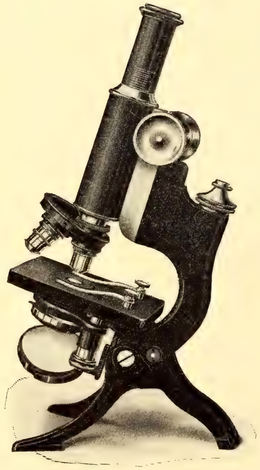

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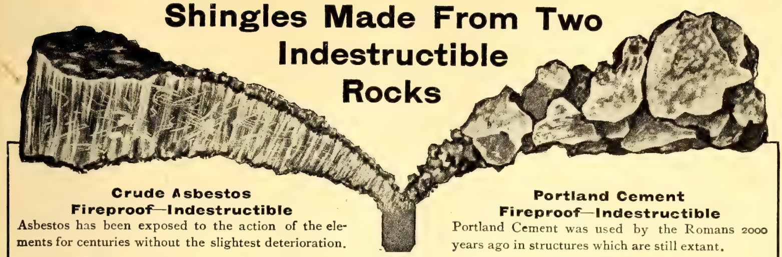

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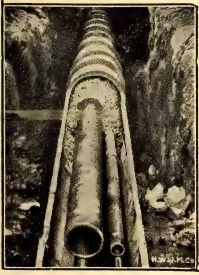

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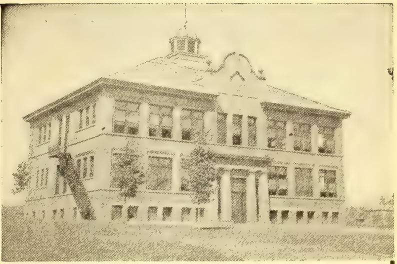

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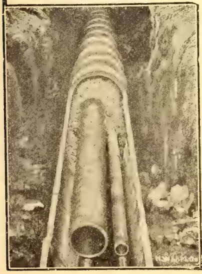

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